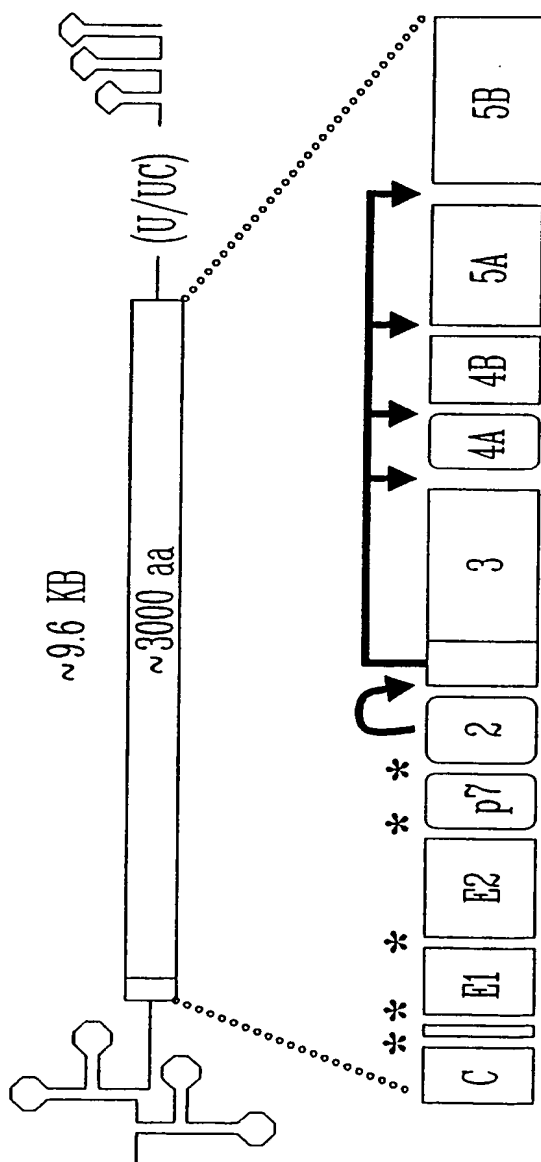


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Hepatitis C virus (HCV) genome organization.

FIG. 1

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Hypothetical model of the HCV replication cycle

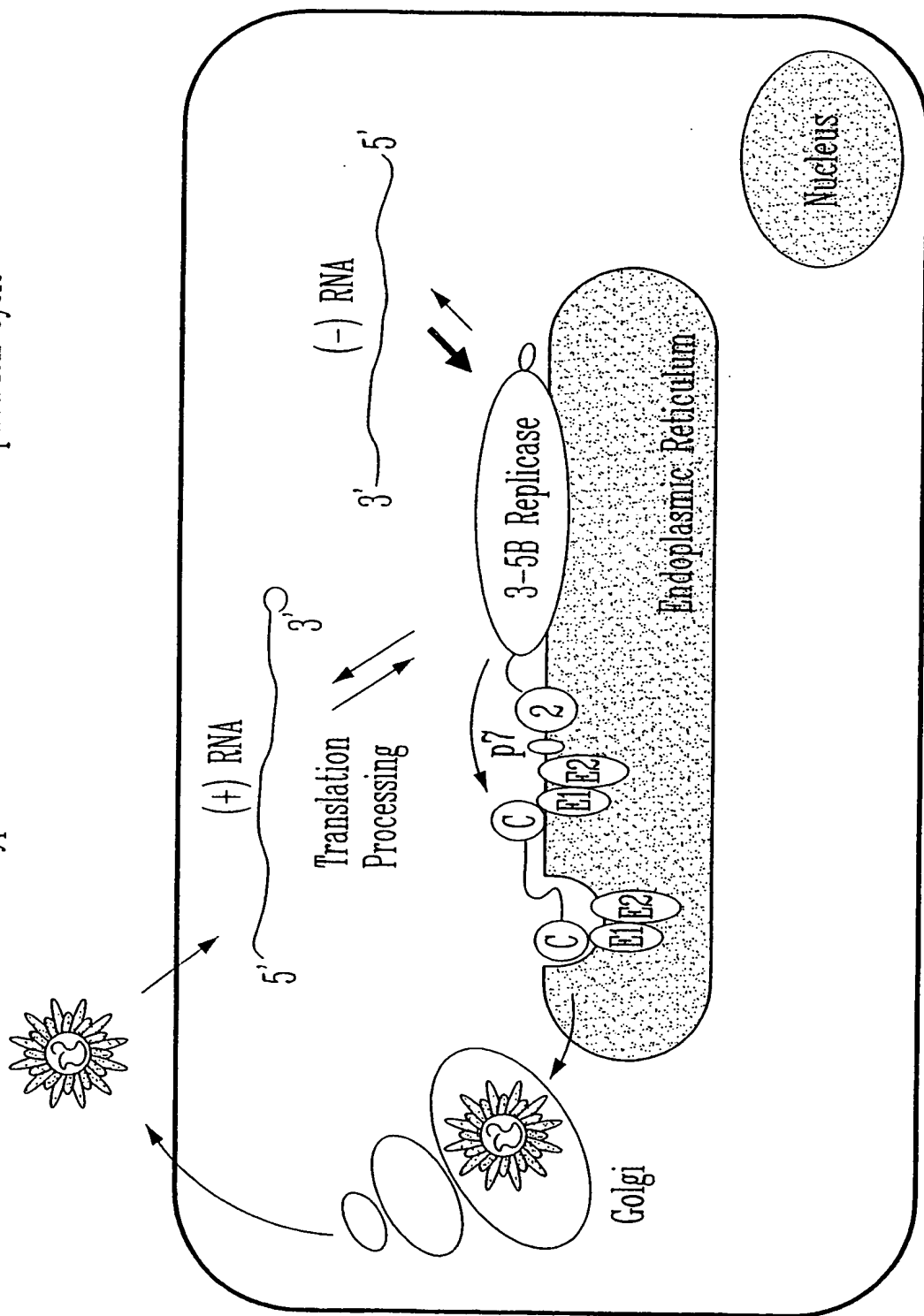


FIG. 2

Experimental Protocol.

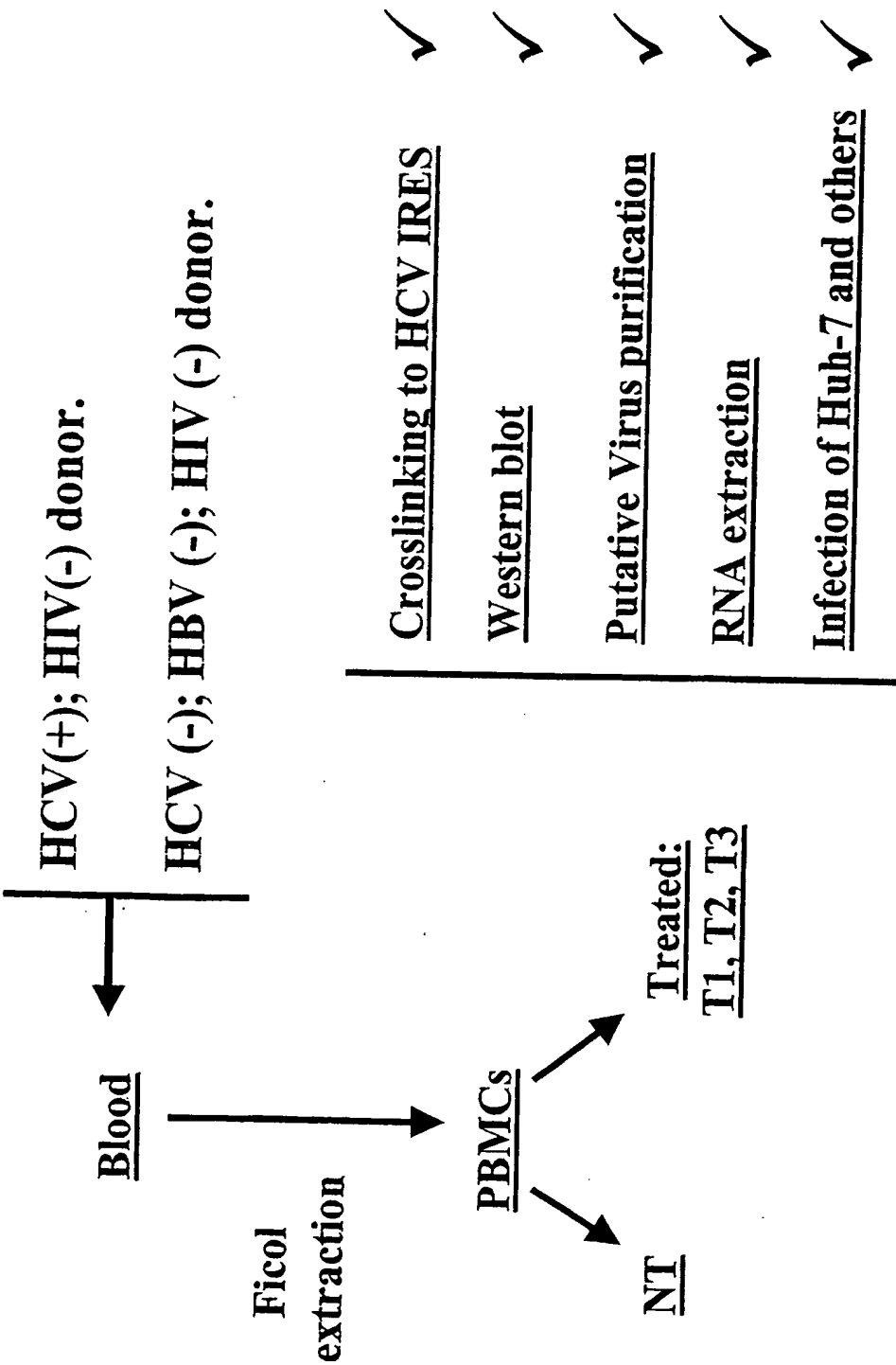
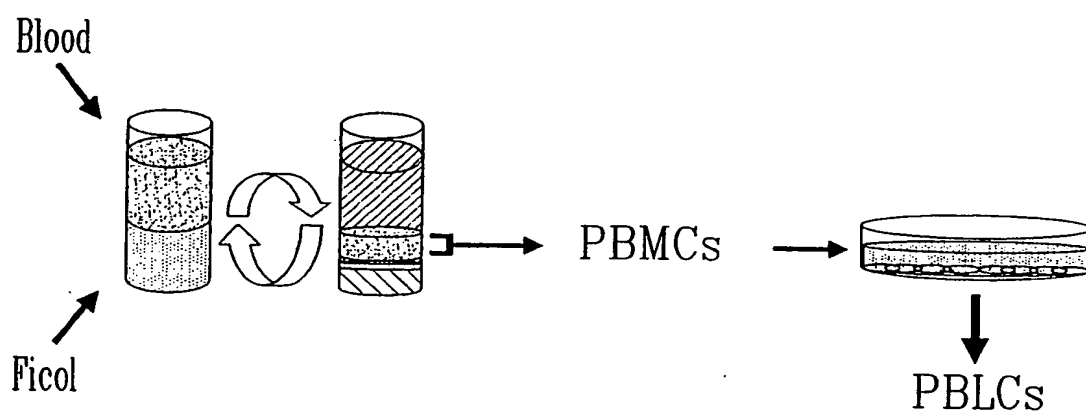


FIG. 3

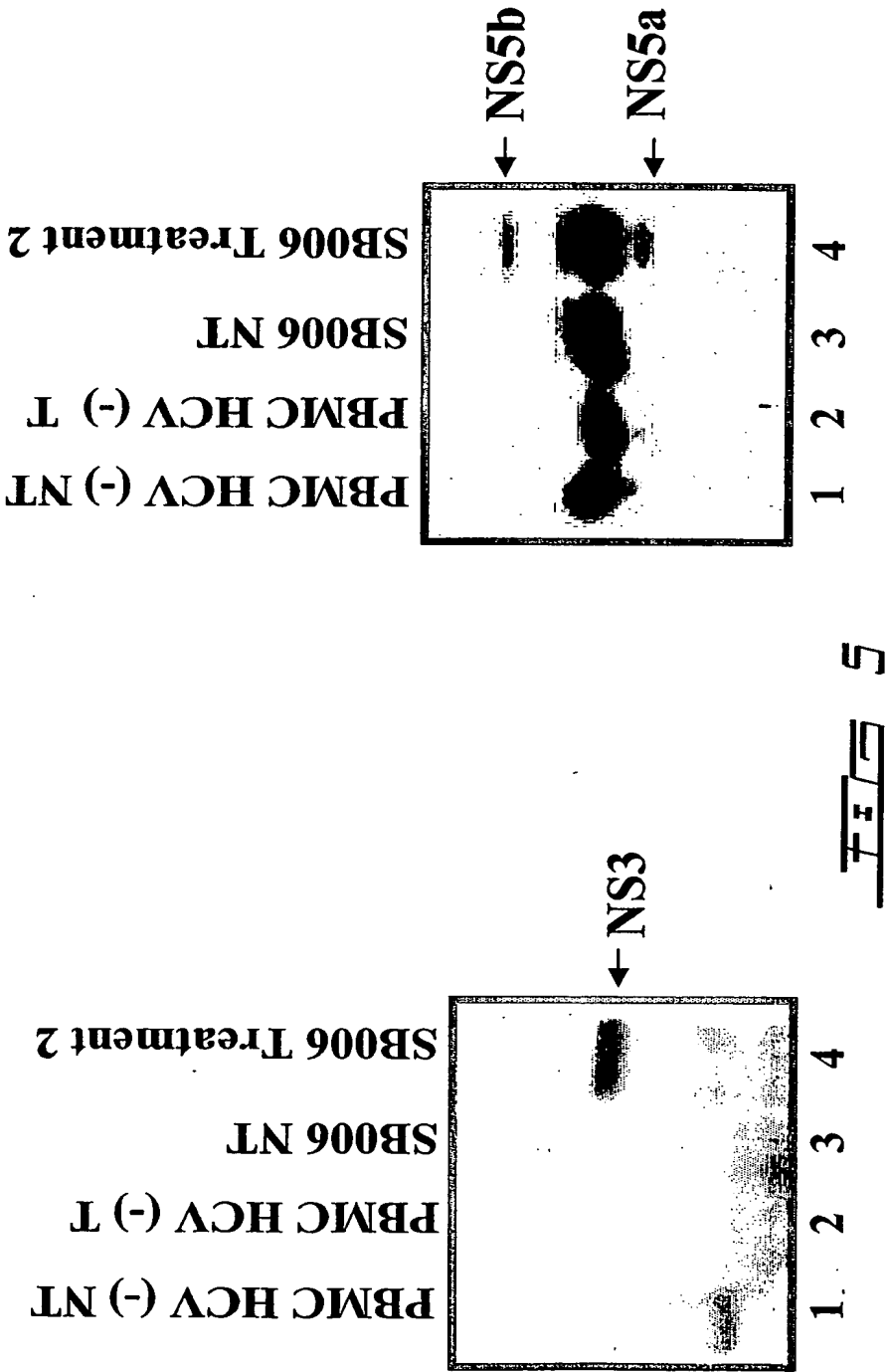
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PBMC and PBLc purification from blood samples.

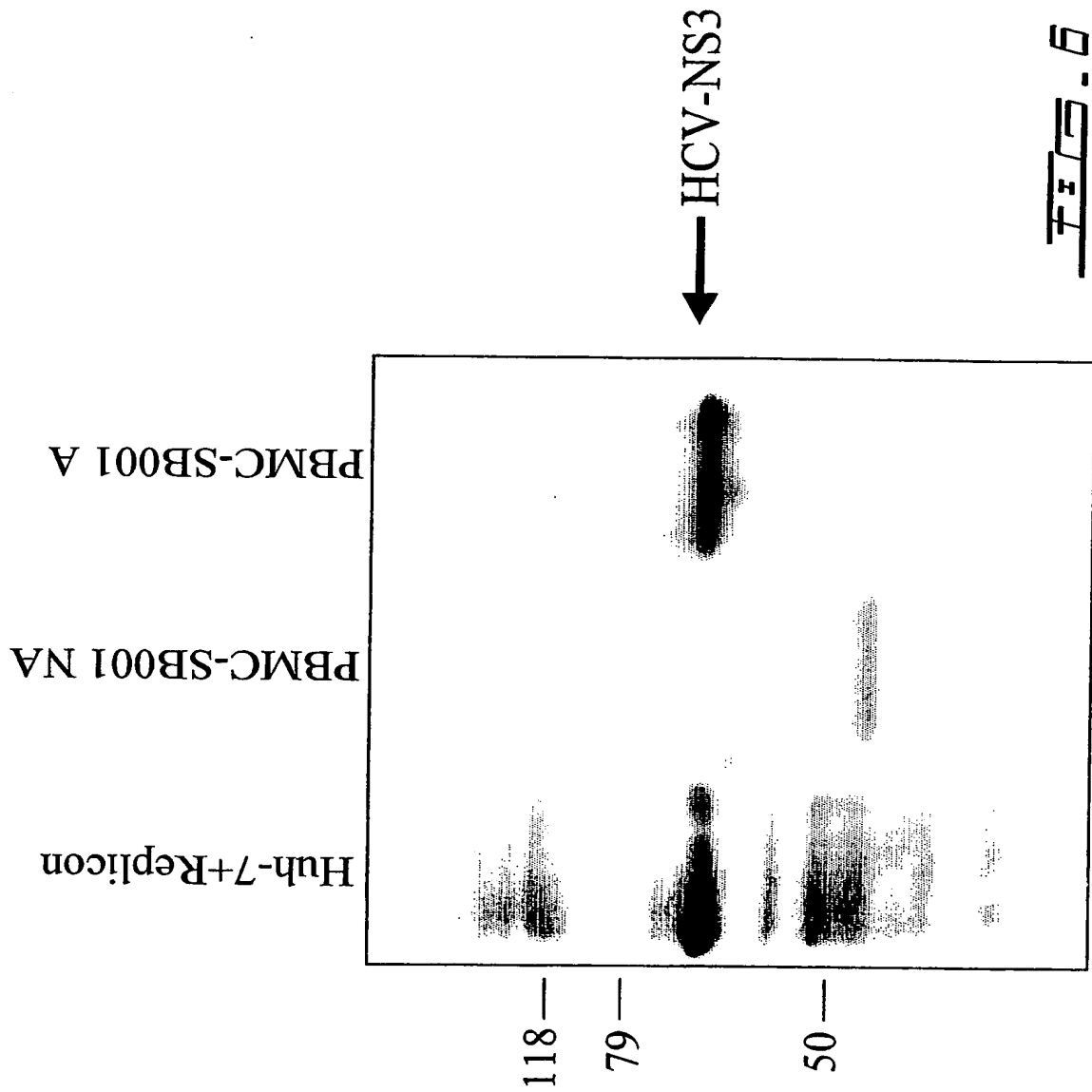
FIG. 4

Detection of HCV NS3 and NS5 proteins in cell extracts from Treated

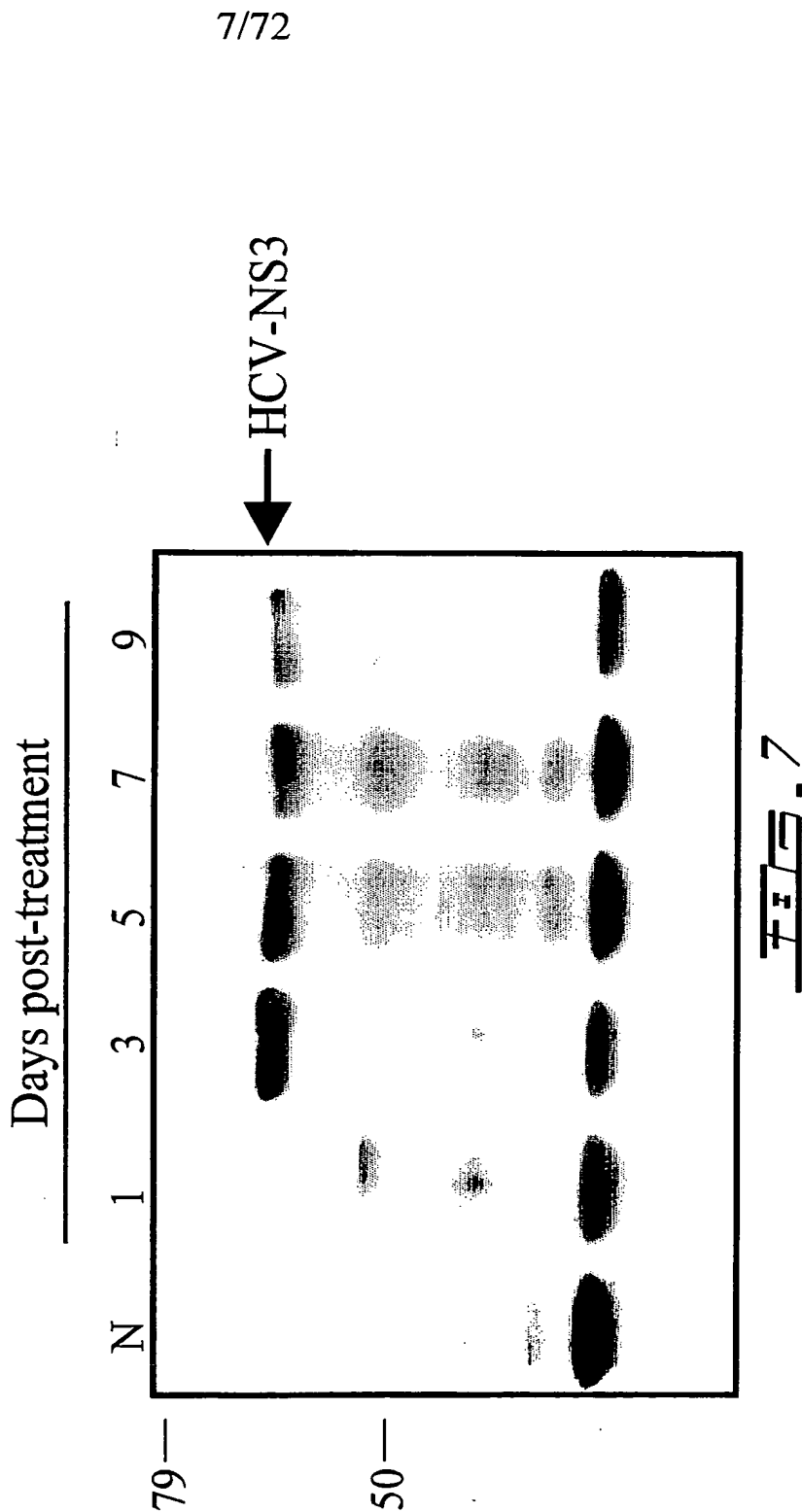
PBMC from an HCV (+) patient.
[Boeringanti-NS3 polyclonal antibody]



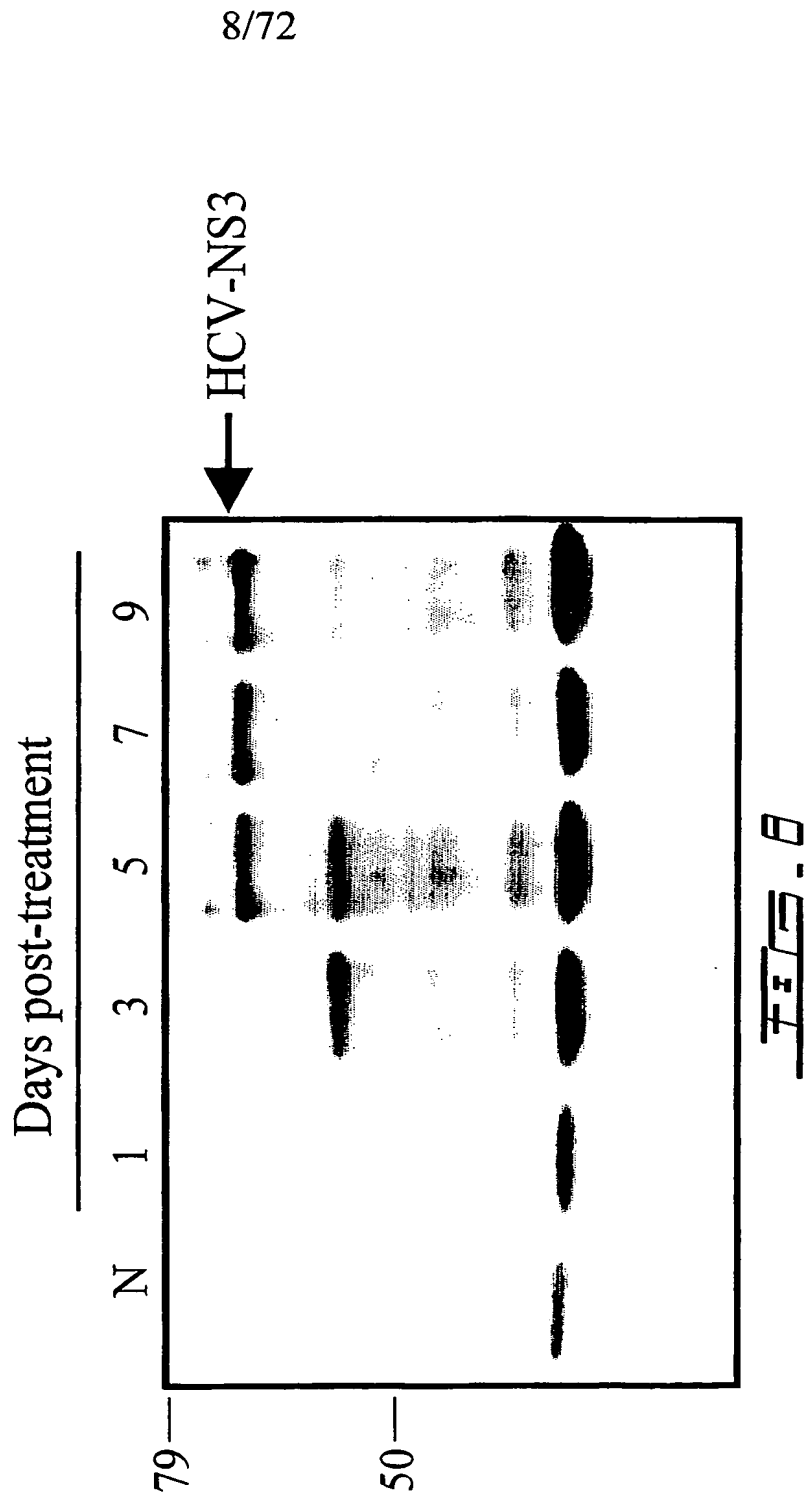
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Time course of HCV-NS3 detection: PBMCs From patient MILL-001



Time course HCV-NS3 detection: PBMCs from patient MLL-002

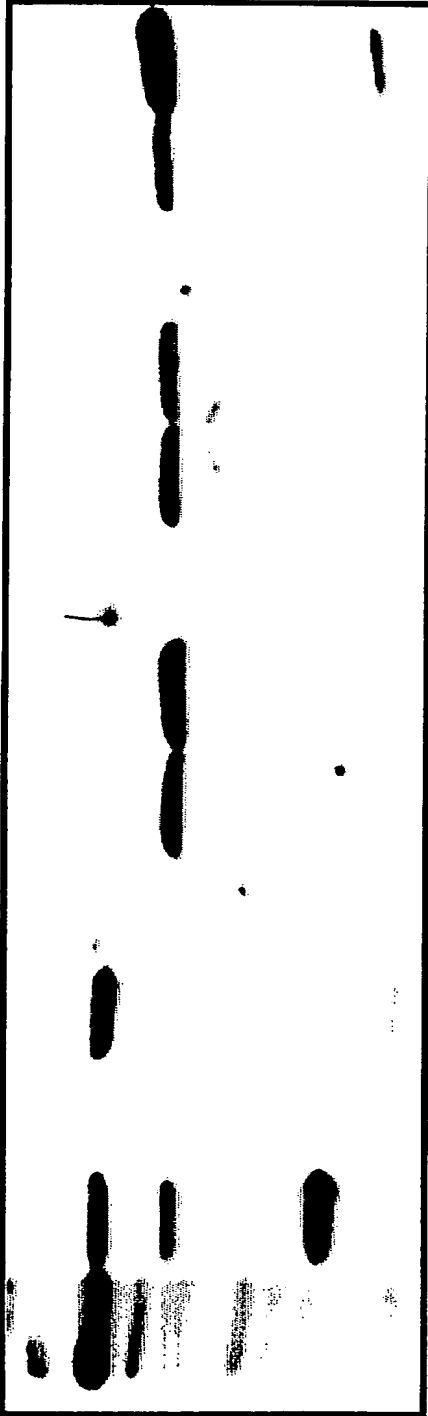


Detection of HCV-NS3 protein in treated (N3) PBMCs from HCV9+) donors

PBMCs
HCV (-) donor

Huh-7
Huh-7+ replicon

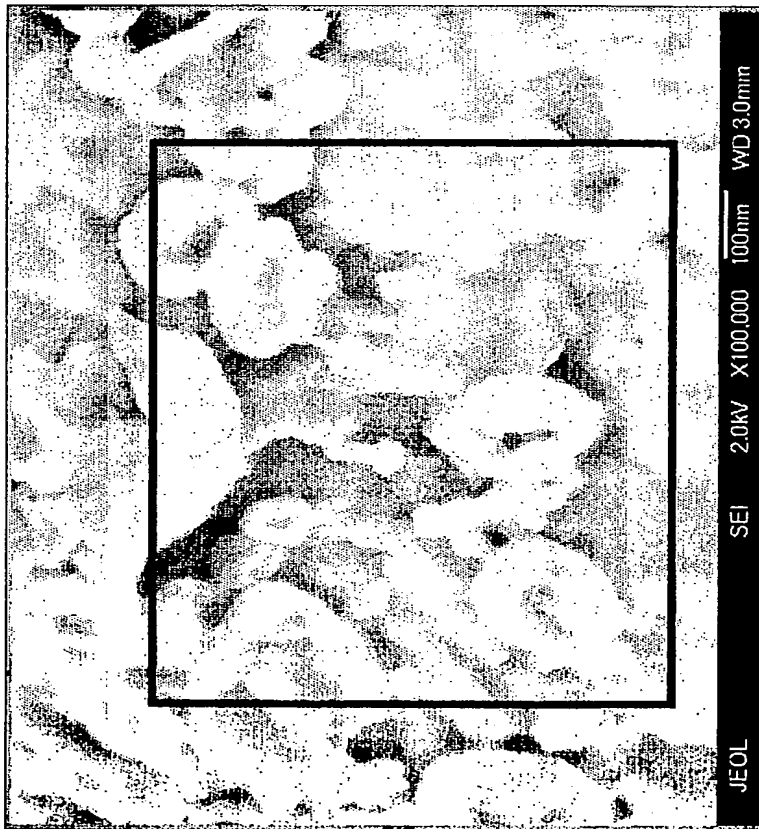
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PBMCs SB006			PBMCs SB004			PBMCs SB005		
N	A	A2	N	A1	A2	N	A1	A2
								

HCV-NS3

Fig. 9

Detection of virus like particles by scanning electron microscopy

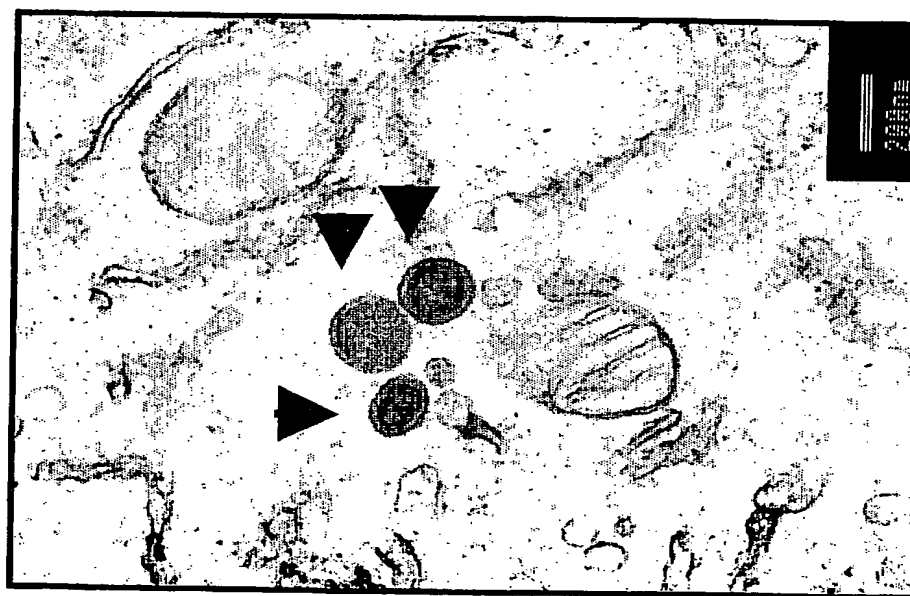


(-) Control

FEI-10

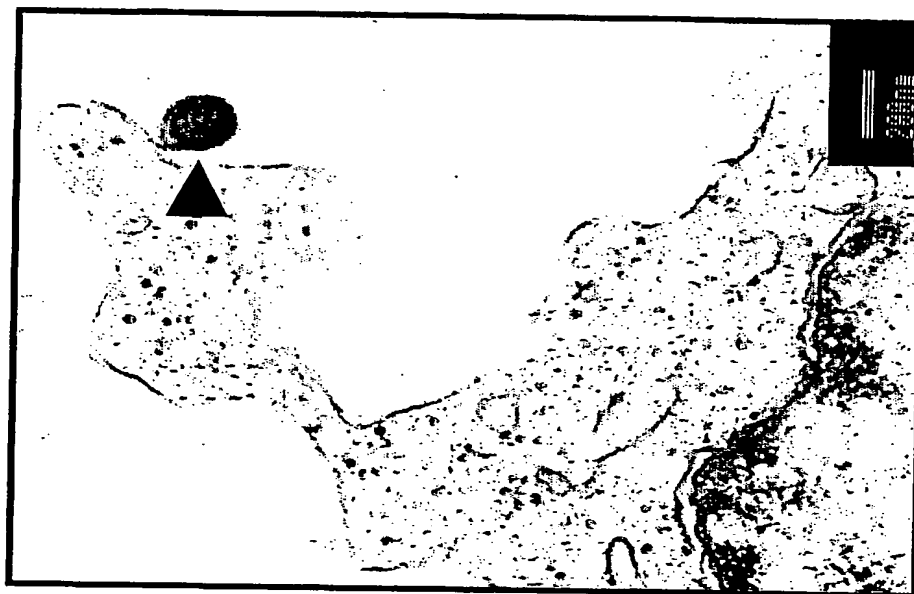
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**Electron microscopy of Activated PBLs;
Detection of virus like particles**



200 nm

Figure 11



200 nm

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Virus partial purification.

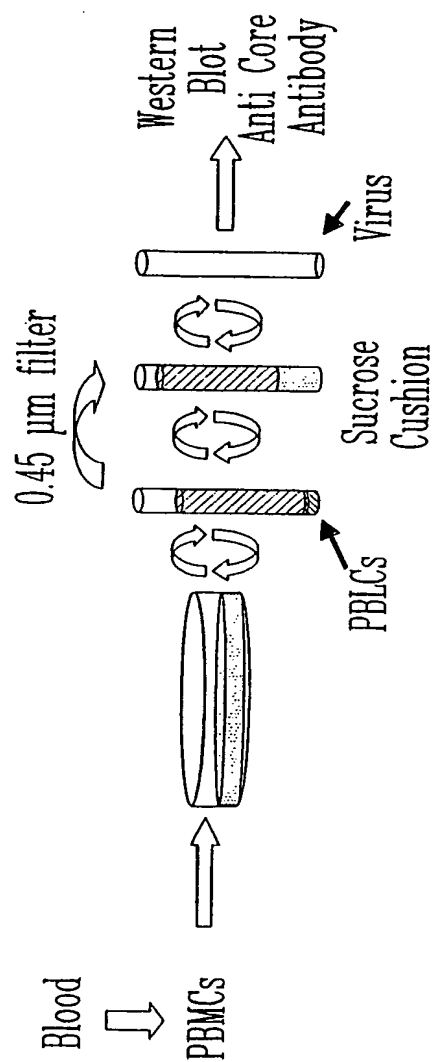
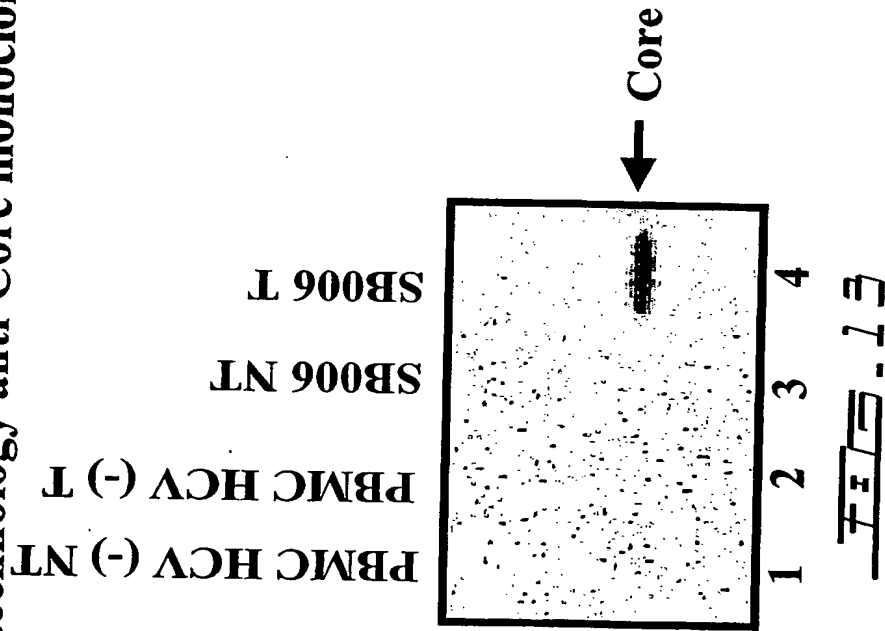


FIG. 12

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**Detection of HCV Core protein in supernatant of treated
PBMC from an HCV (+) patient.
[Maine biotechnology anti-Core monoclonal antibody]**



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RNA Quantification I (virus copies/ng total RNA)

Patient
After 4 days

HCV RNA
In PBMC

Detection of
Core (wb) in
supernatant

SB004 NT

2x10³

No

SB004 T

2x10³

Yes

SB006 NT

1.8 x10³

No

SB006 T

2x10²

Yes

After 20 days

SB004

0.00

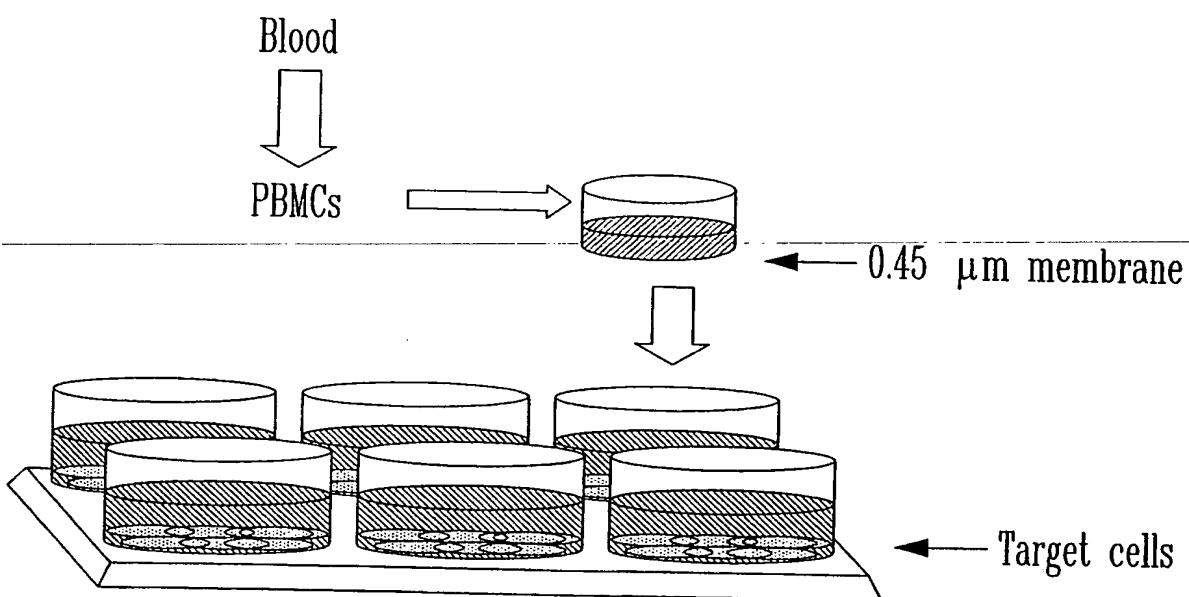
SB006

0.00

7113 - 14

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Infection assay; co-culture

FIG. 15

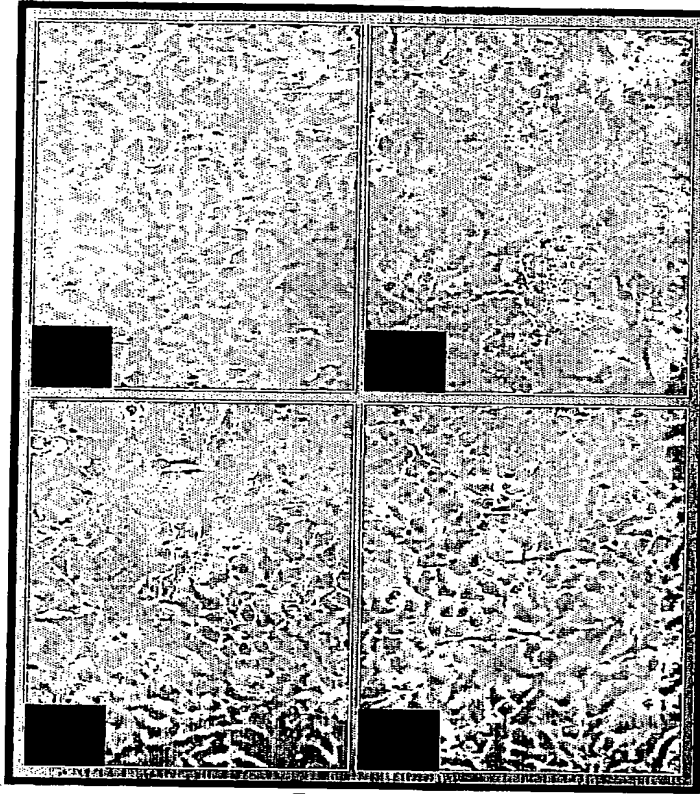
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Infection of MT-4 cells RNA Quantification II (virus copies/ng total RNA)			
Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant	HCV RNA In MT-4
<u>After 10 days</u>			
SB001 NT	13	No	0.00
SB001 T	12	Yes	1600
<u>After 20 days</u>			
SB001	0.00		0.00
SB001	0.00		0.00

~~TABLE - 15~~

Co-culture of Huh-7 and HCV (-) PBMCs.

- 1- Huh-7
- 2- Huh-7 + PBMCs HCV (-) NT
- 3- Huh-7 + Treatment
- 4- Huh-7 + PBMCs HCV (-) T

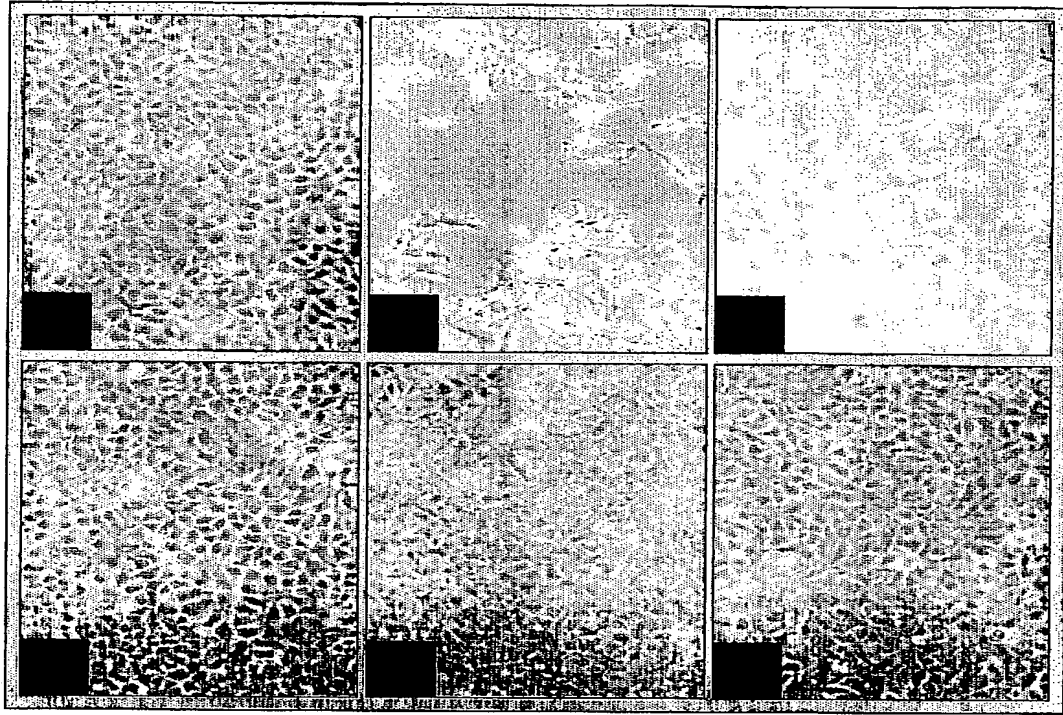


FILE - 17

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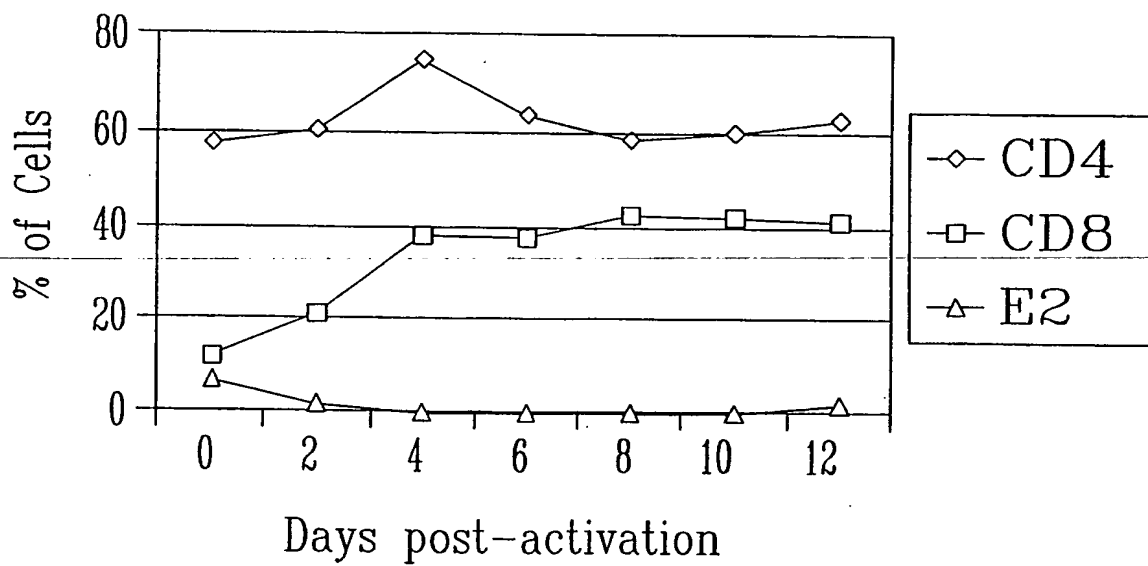
Co-culture of Huh-7 and HCV (+) PBMS^o Cs (SB0006).

1. Huh-7
- 2-3. Huh-7 + PBMCs HCV (+) NT
4. Huh-7 + Treatment
- 5-6. Huh-7 + PBMCs HCV (+) T

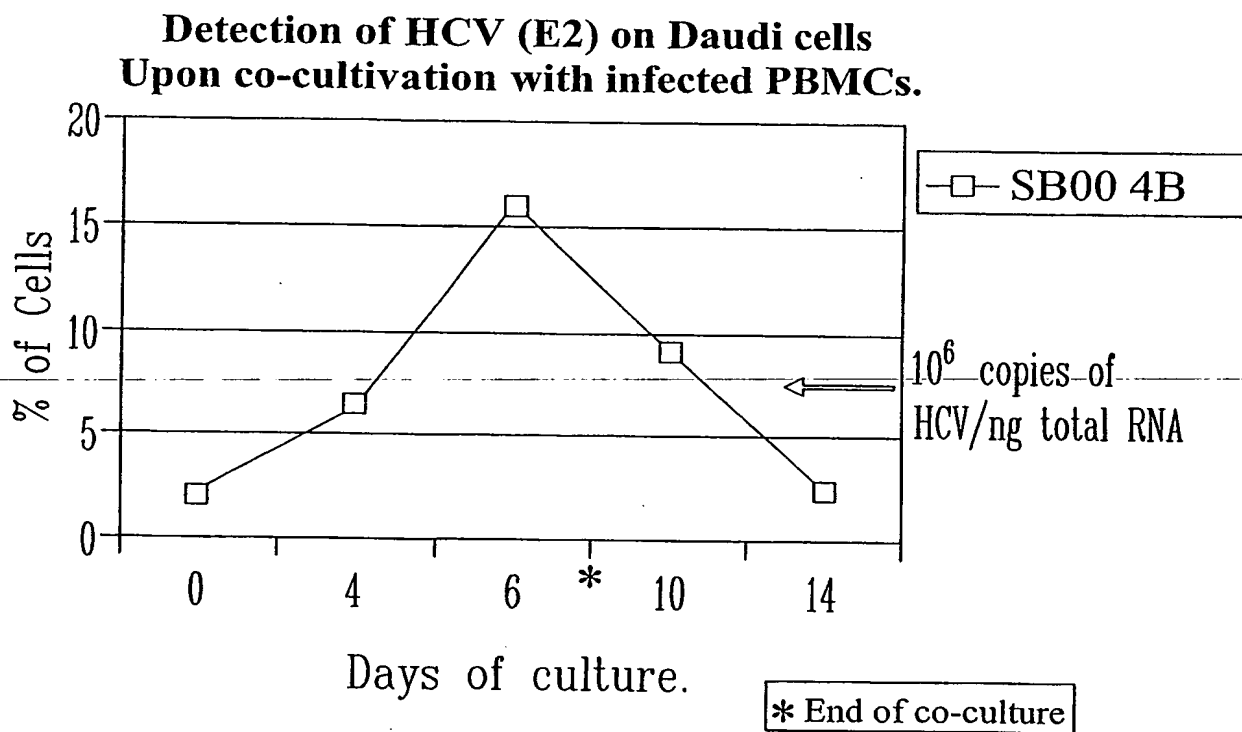
FIG. 18

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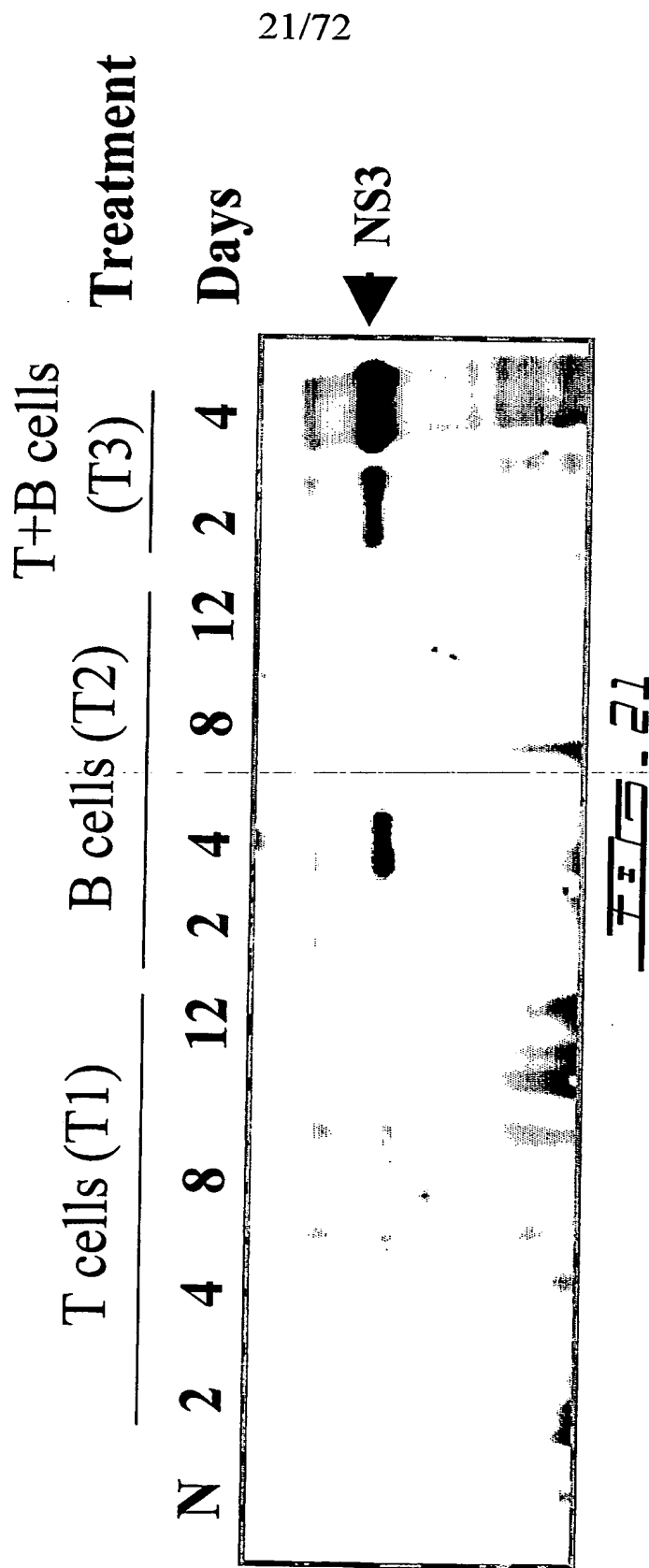
PHA Activation of PBMCs from patient SB004;
HCV is not in T cells

FIG. 19

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FIG. 20

**Comparison of different activation treatments;
PBMCs from donor MLL-010**



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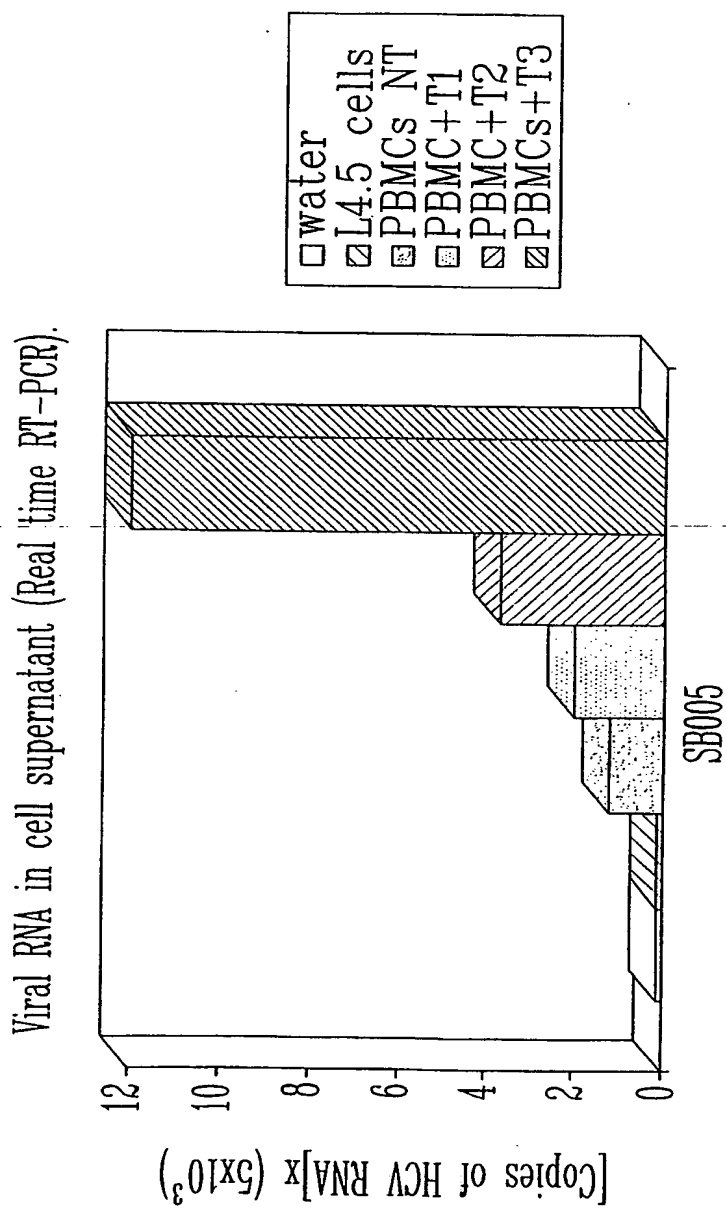


FIG. 22

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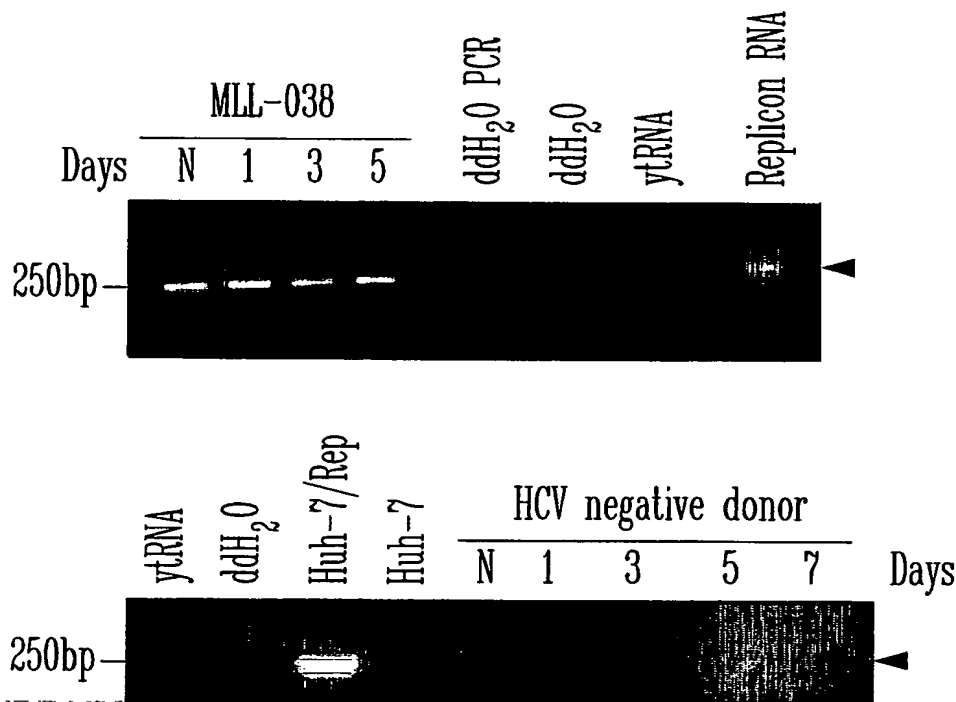


FIG. 23 A

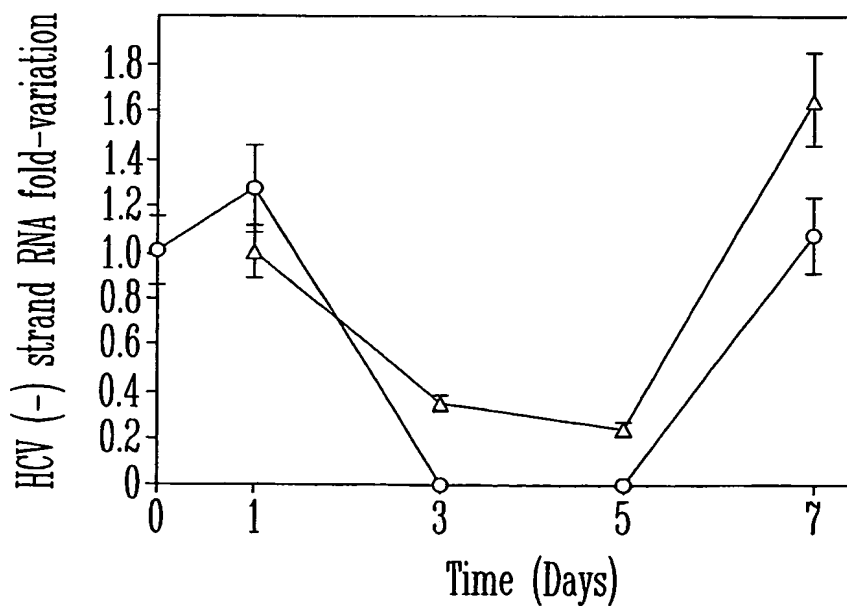


FIG. 23 B

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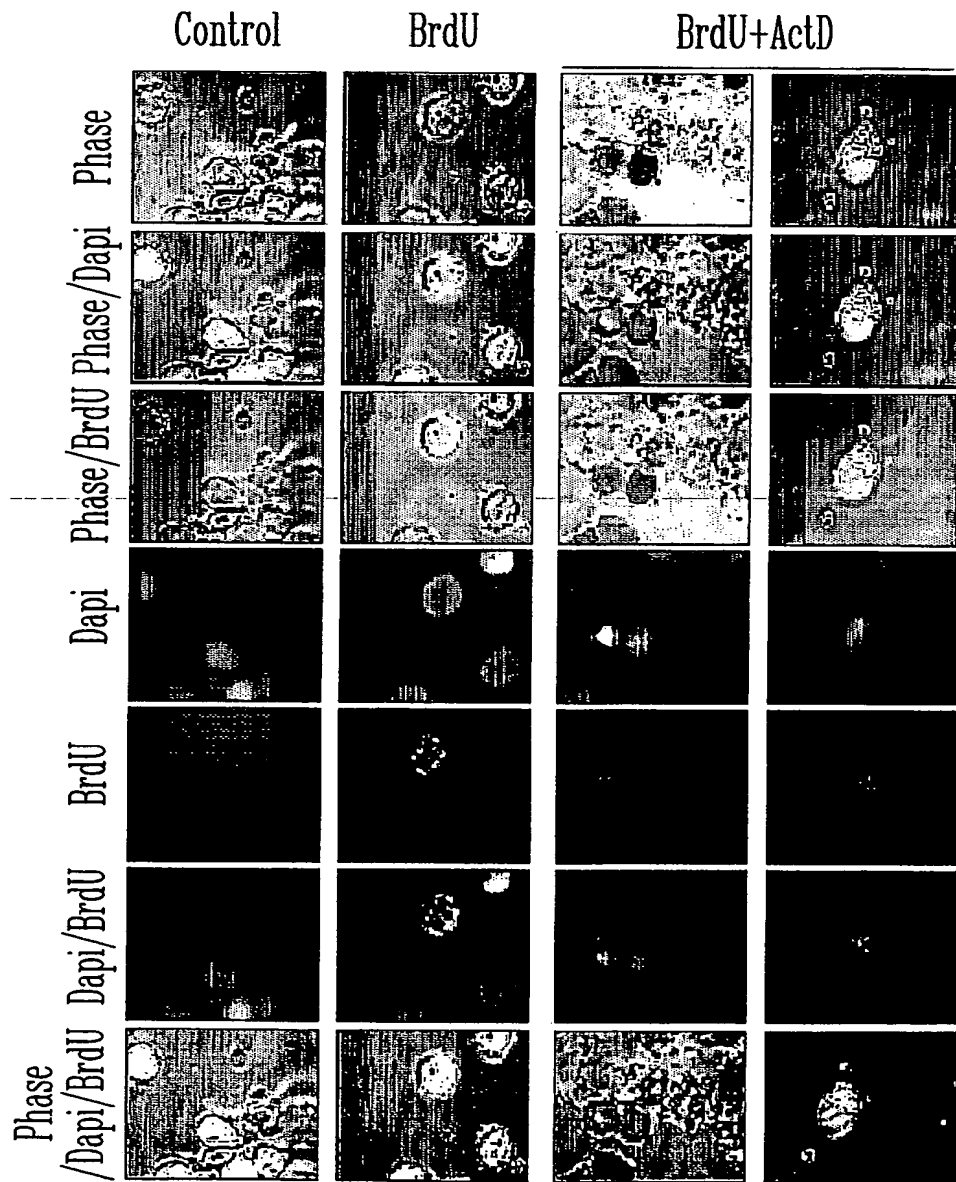


FIG. 23C

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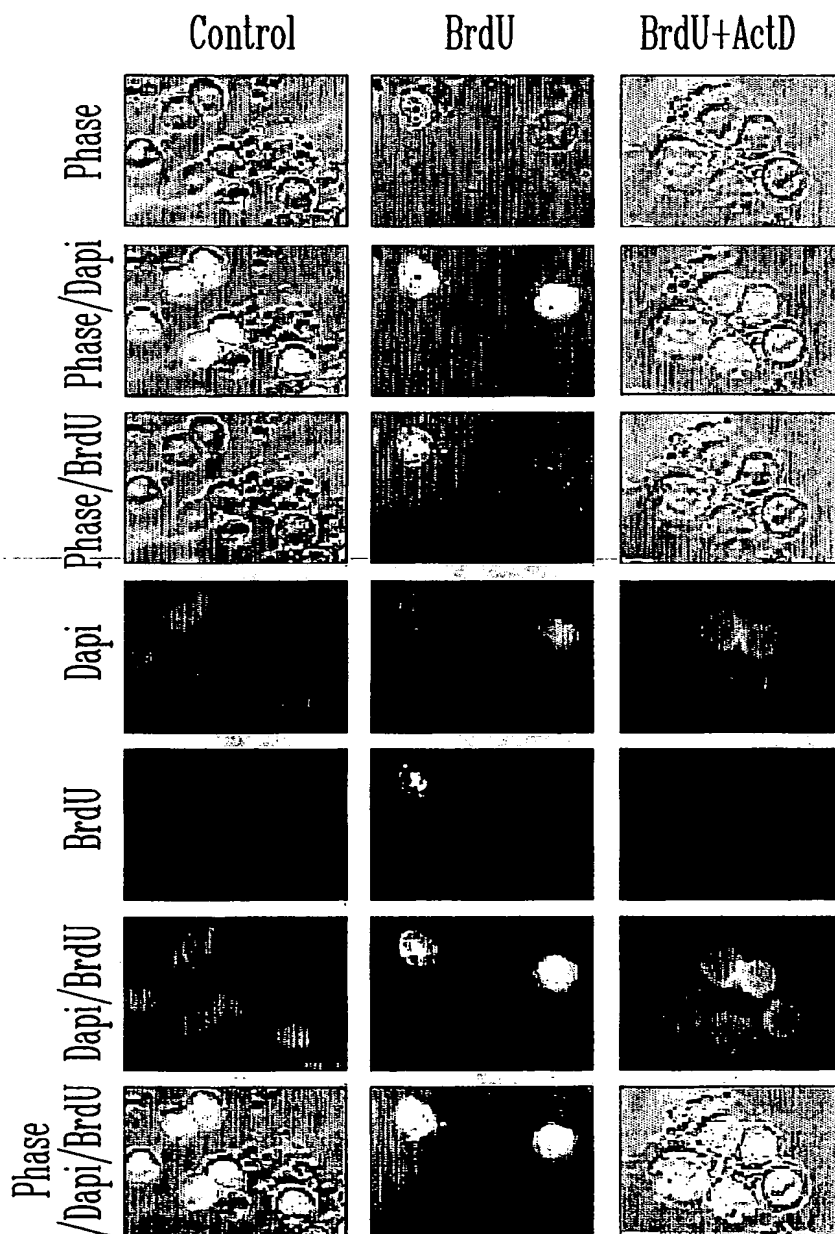
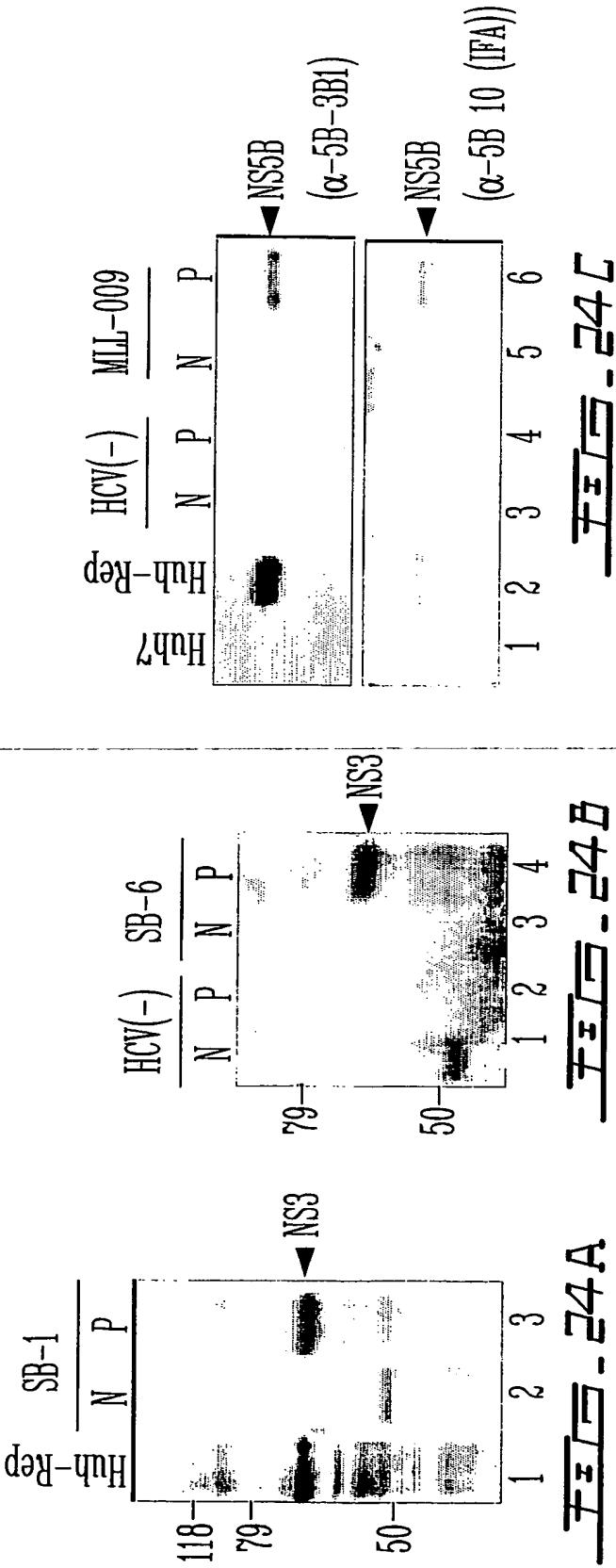
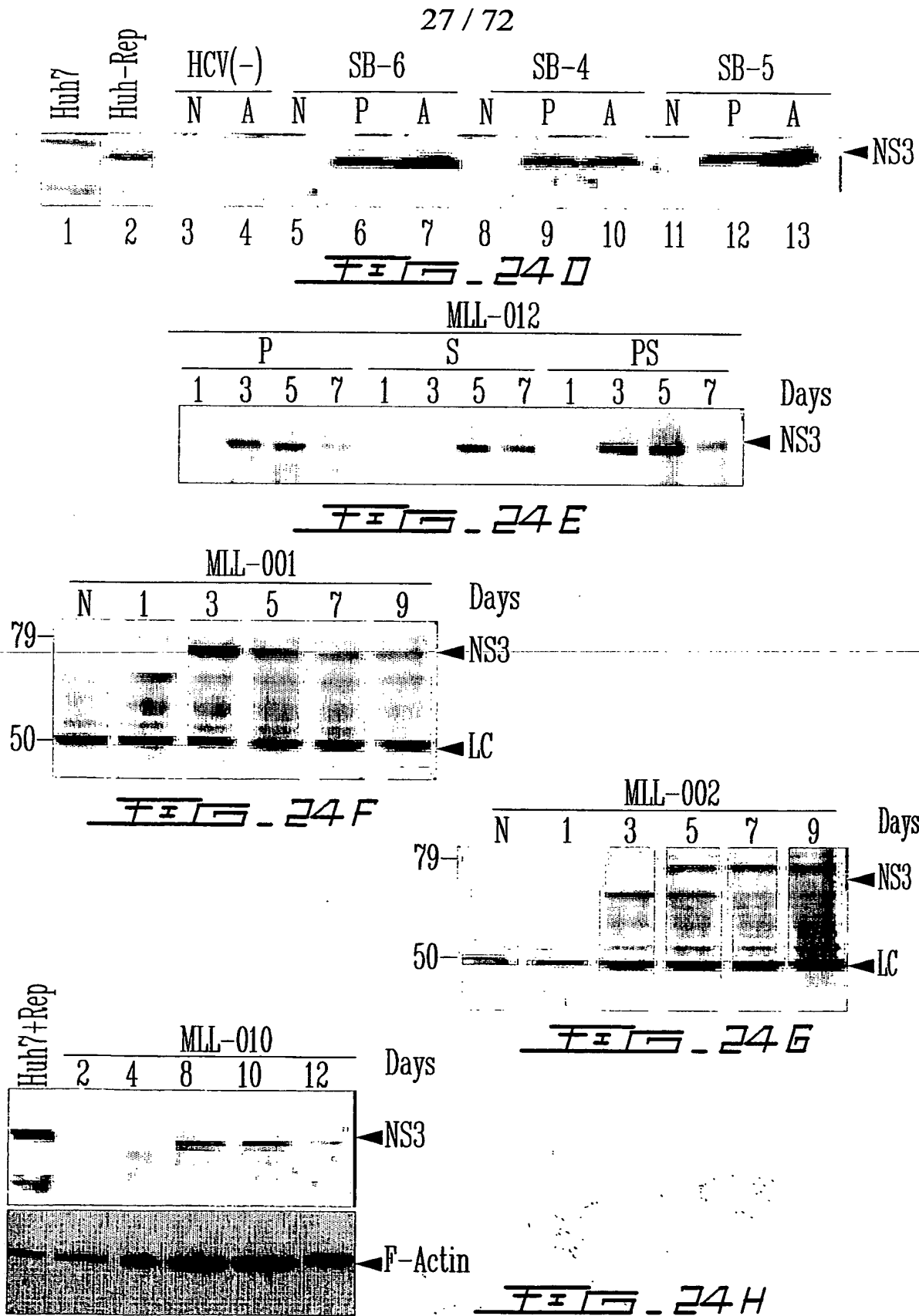


Fig. 23D





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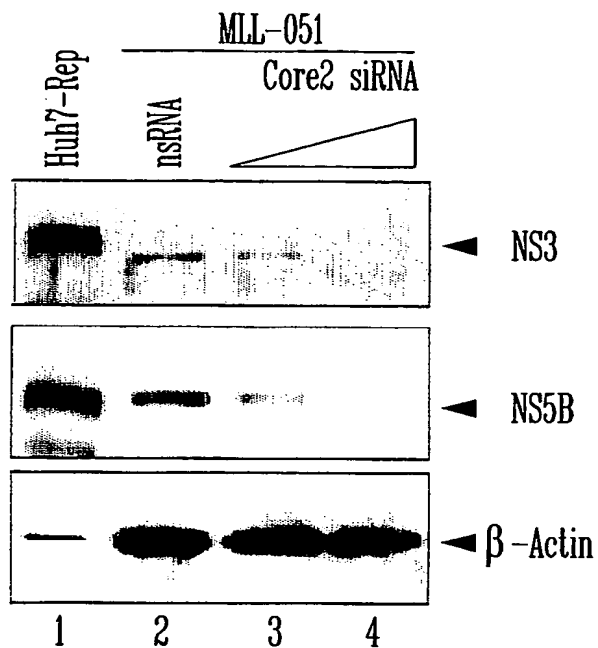


FIG. 24I

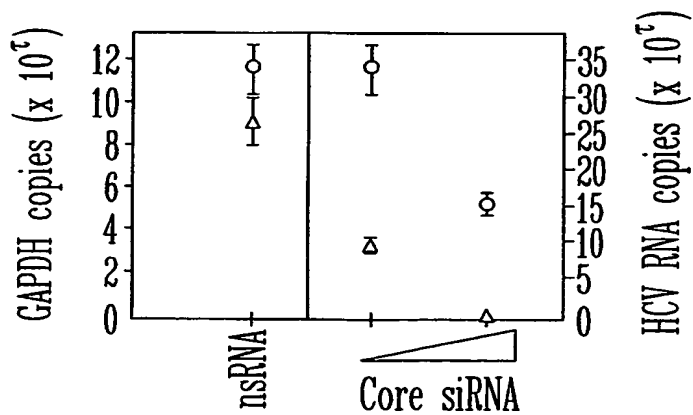
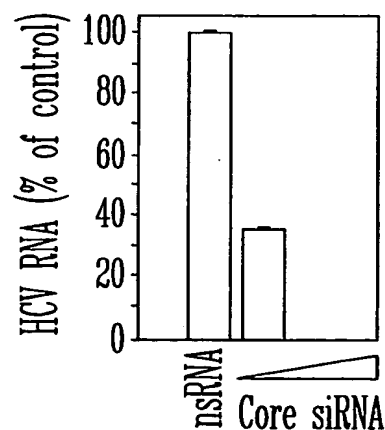


FIG. 24J

FIG. 24K



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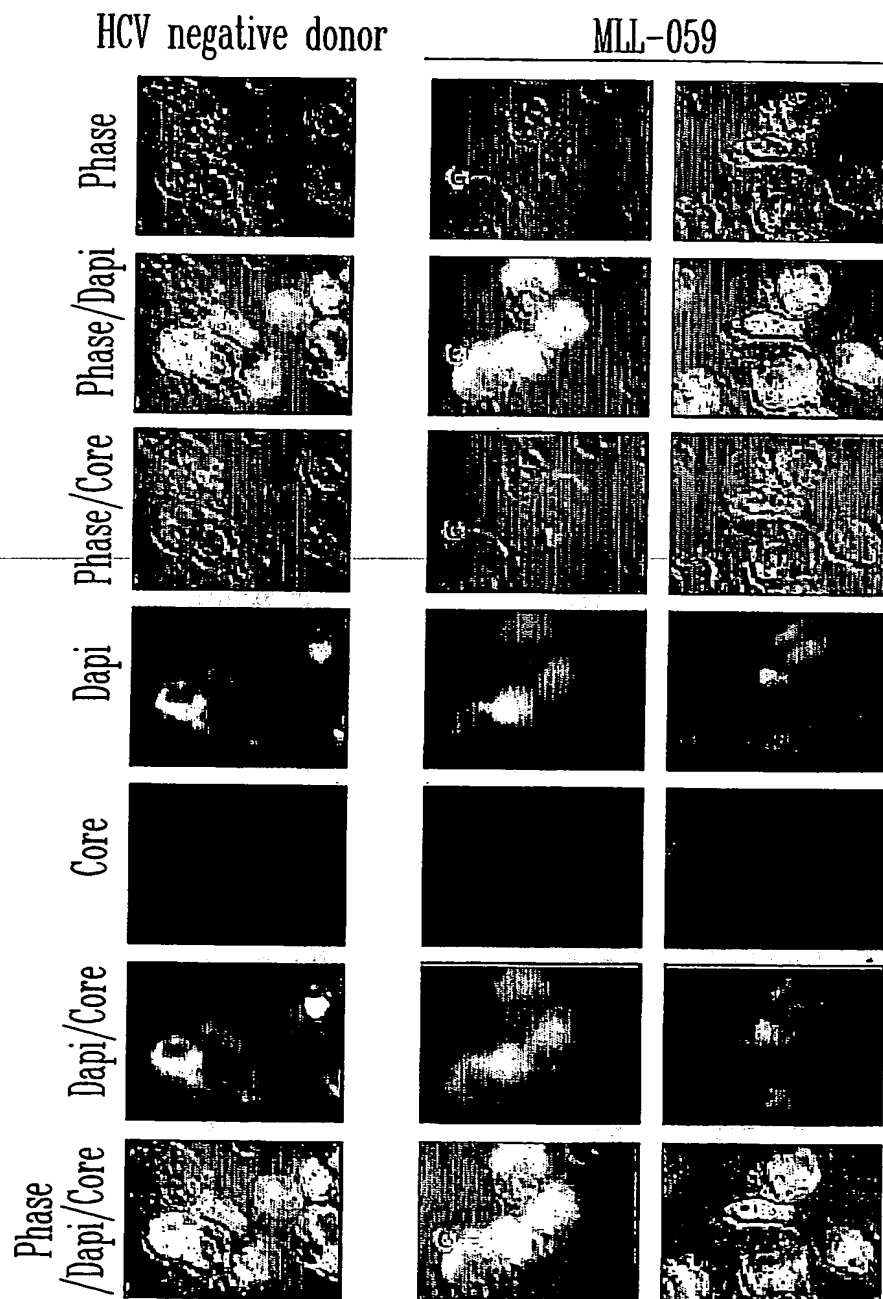


FIG. 25

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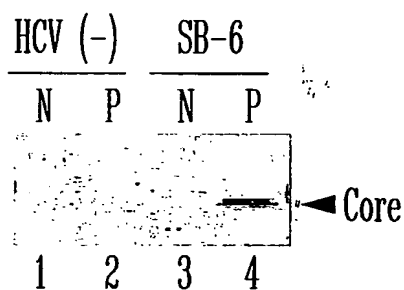


FIG. 26A

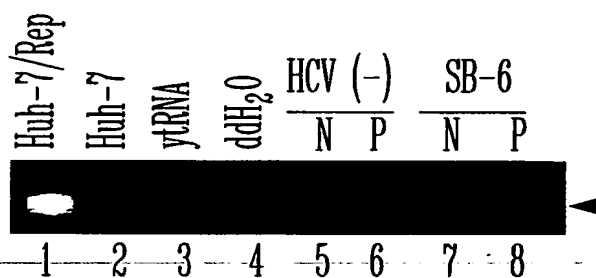
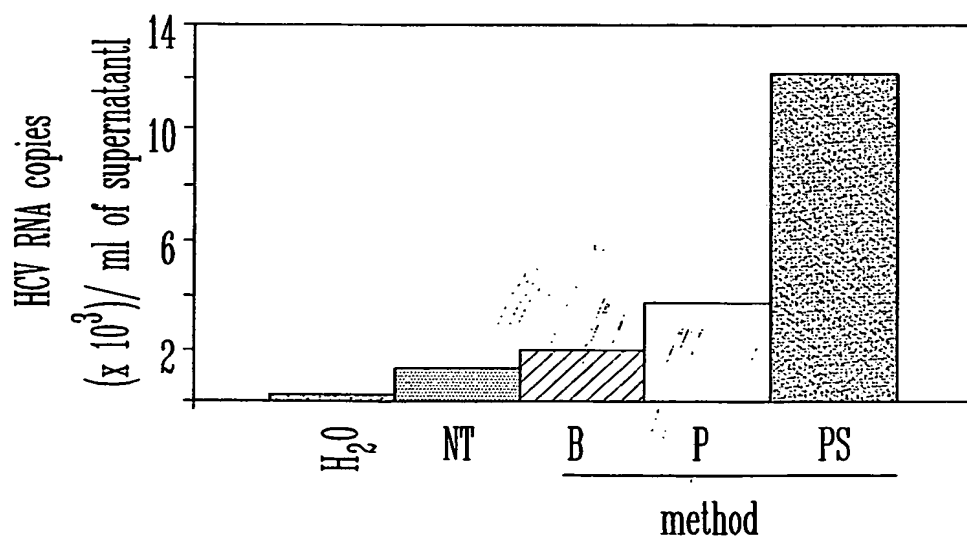


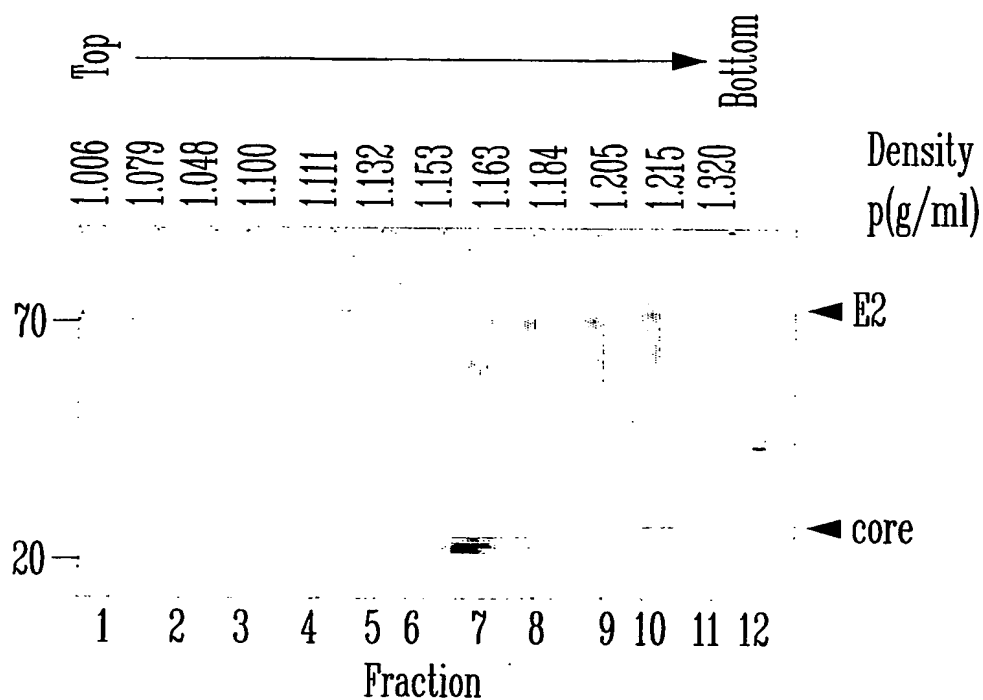
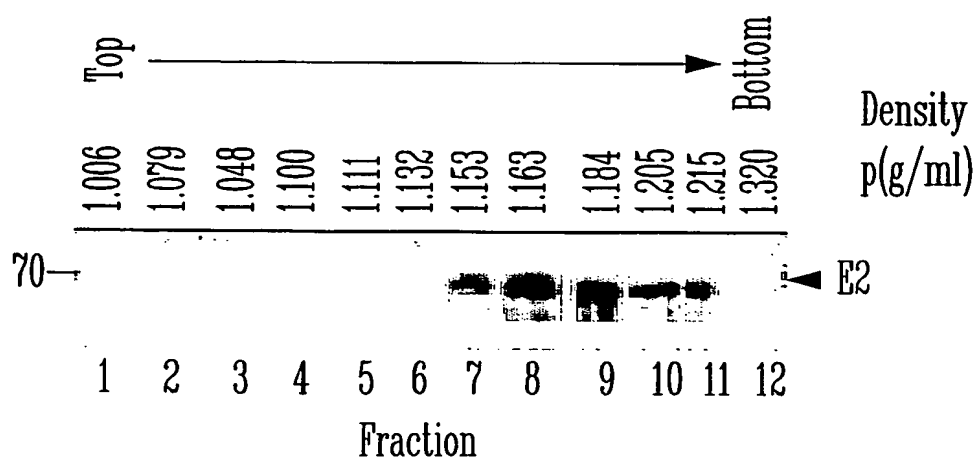
FIG. 26B



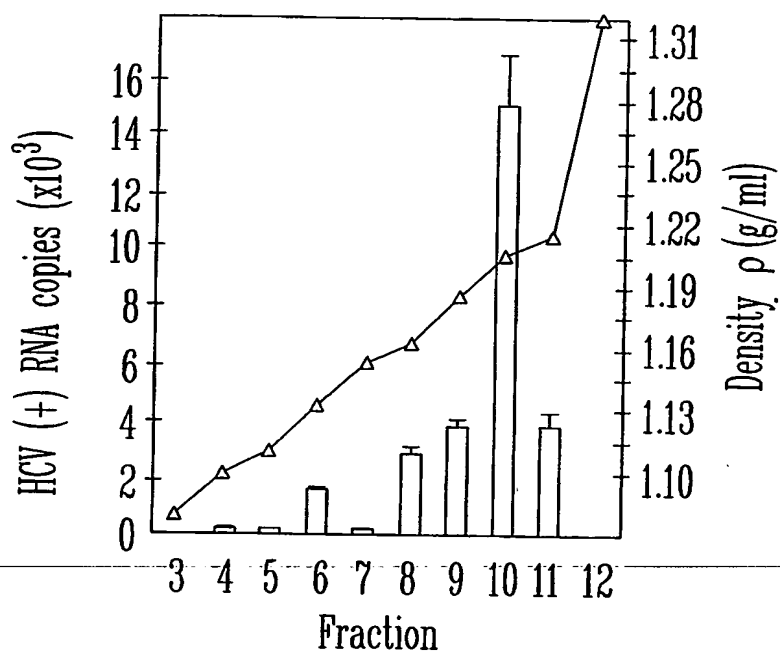
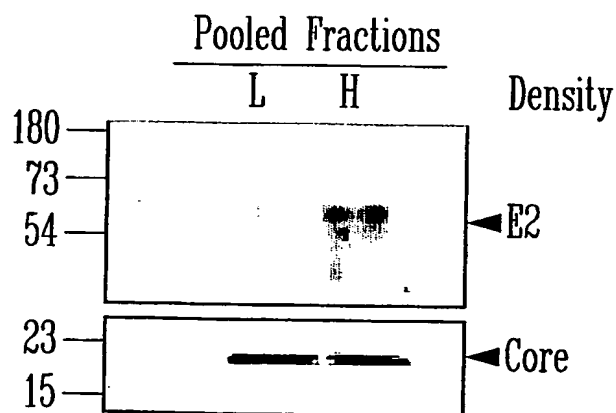
SB-5

FIG. 26C

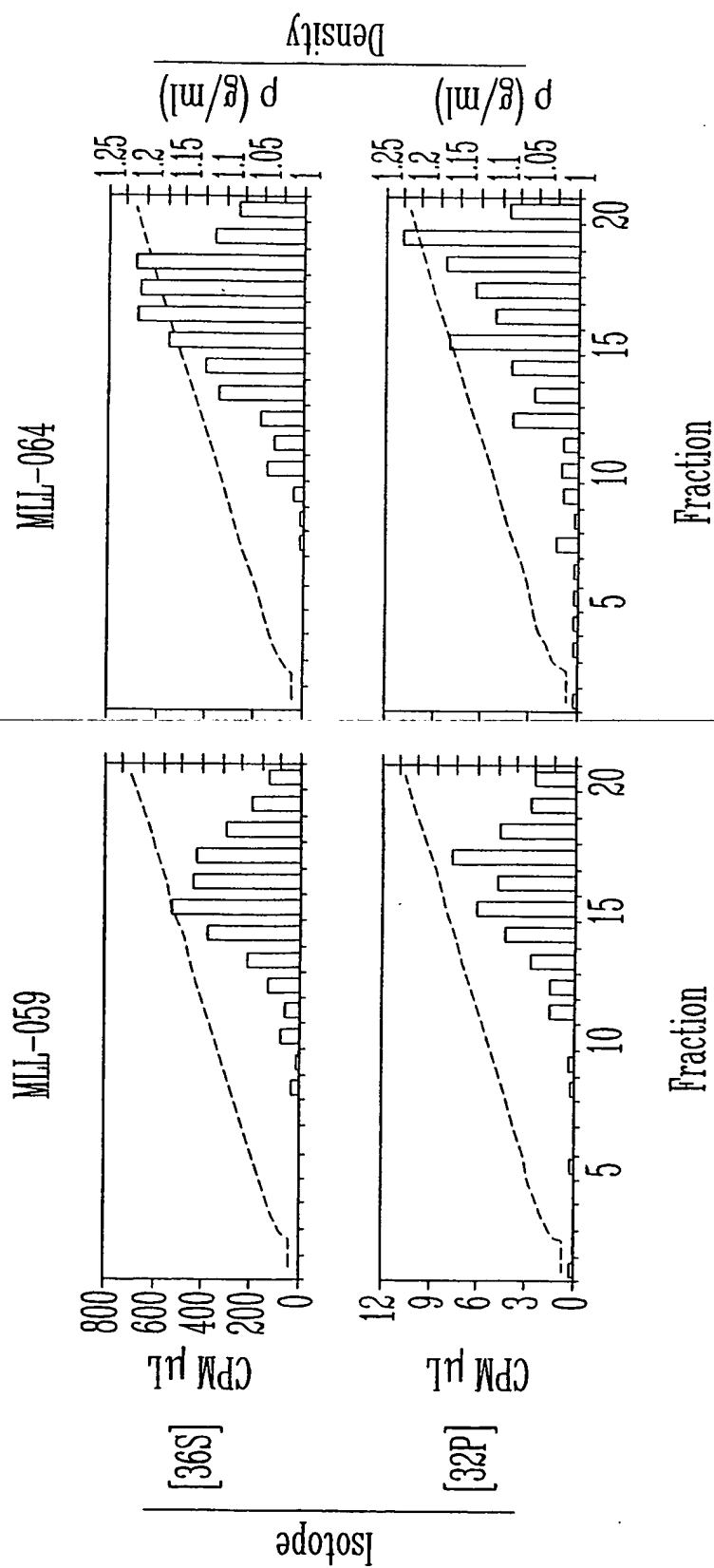
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FIG. 260FIG. 26E

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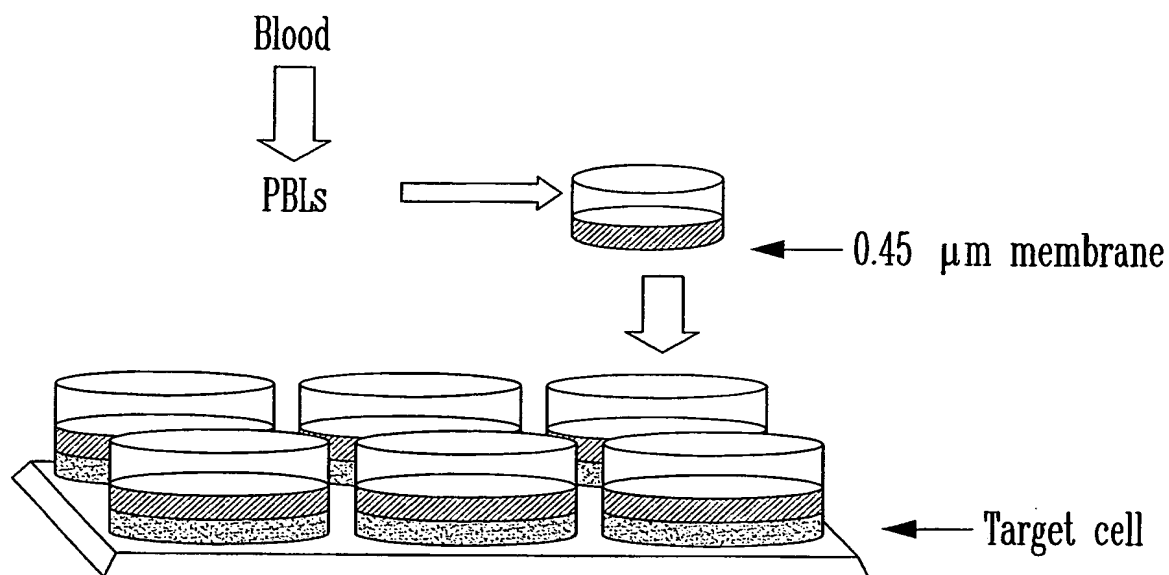
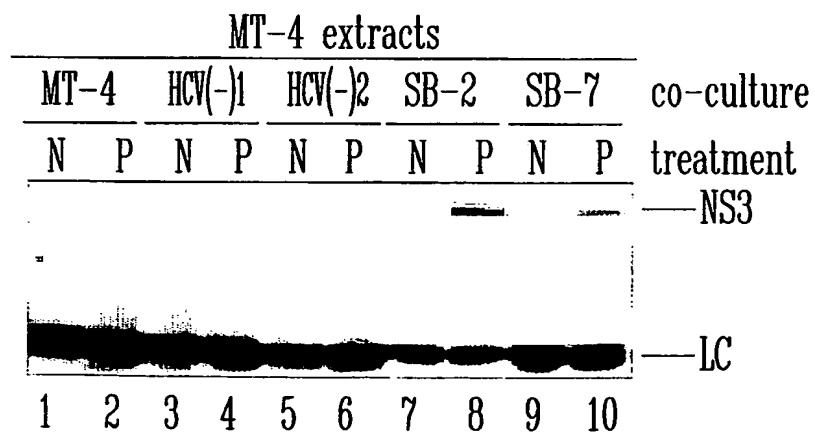
FIG. 26FFIG. 26G

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7310-25H

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FIG. 27AFIG. 27B

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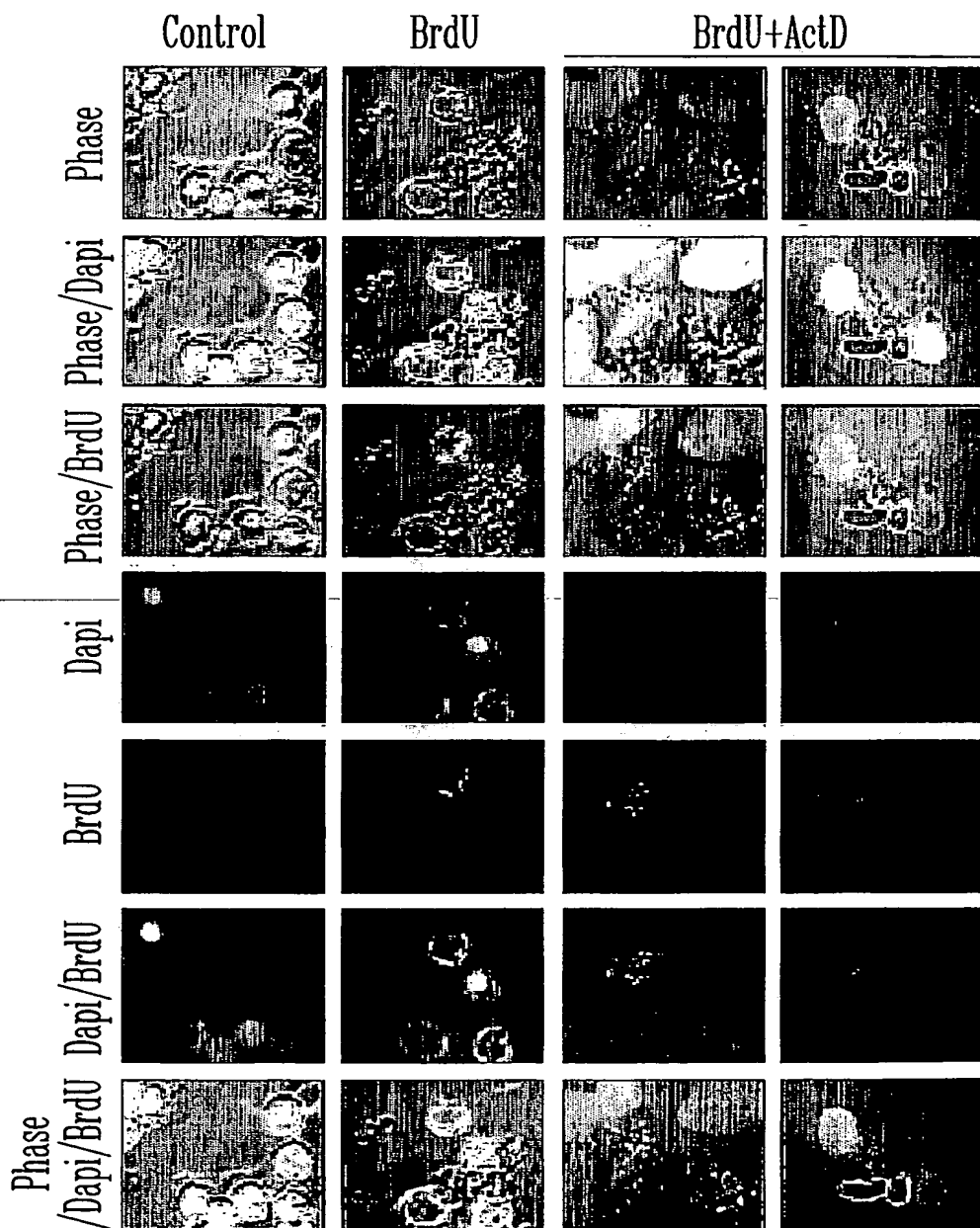
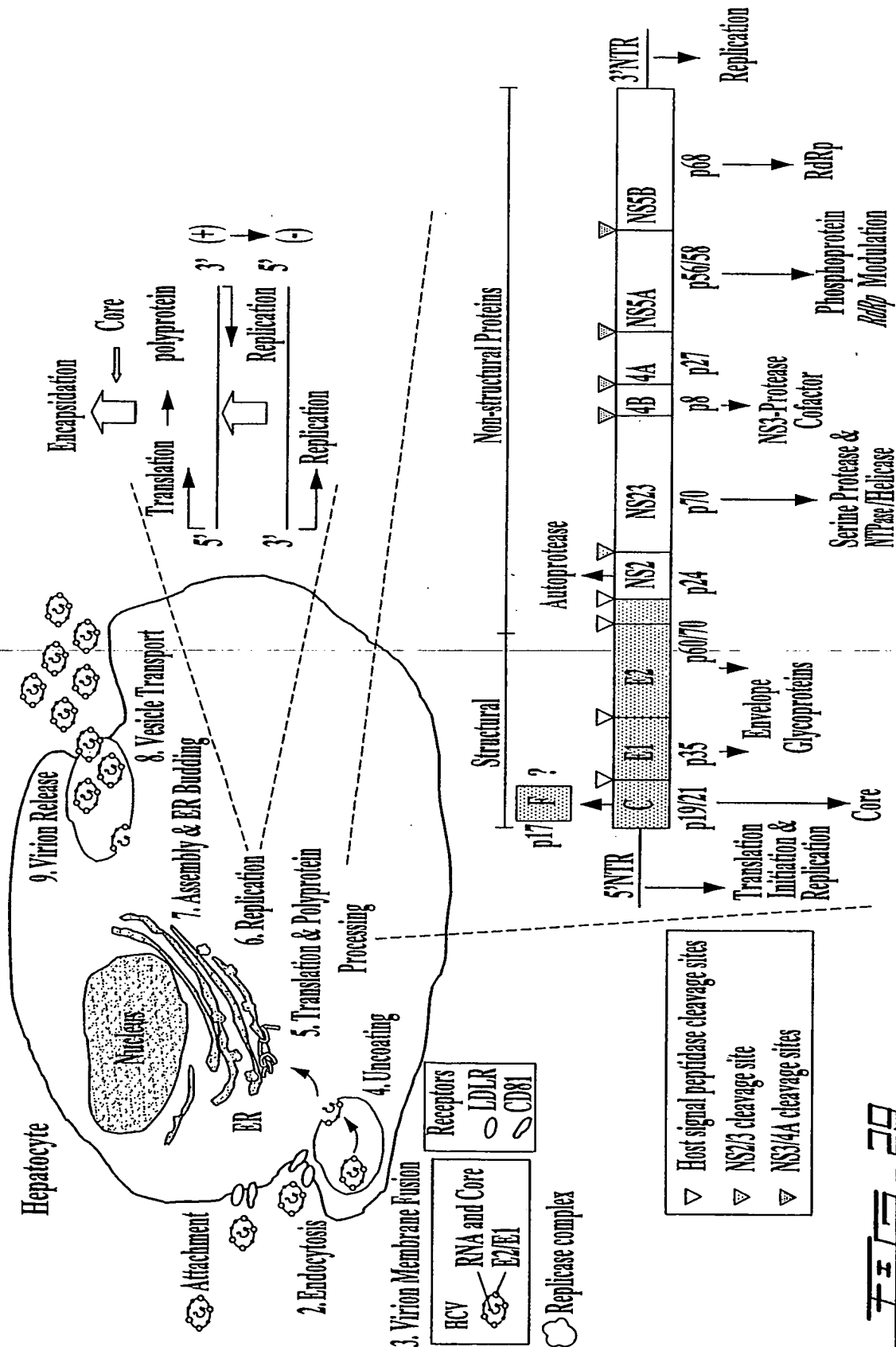


FIG. 2B

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HCV Replication Cycle



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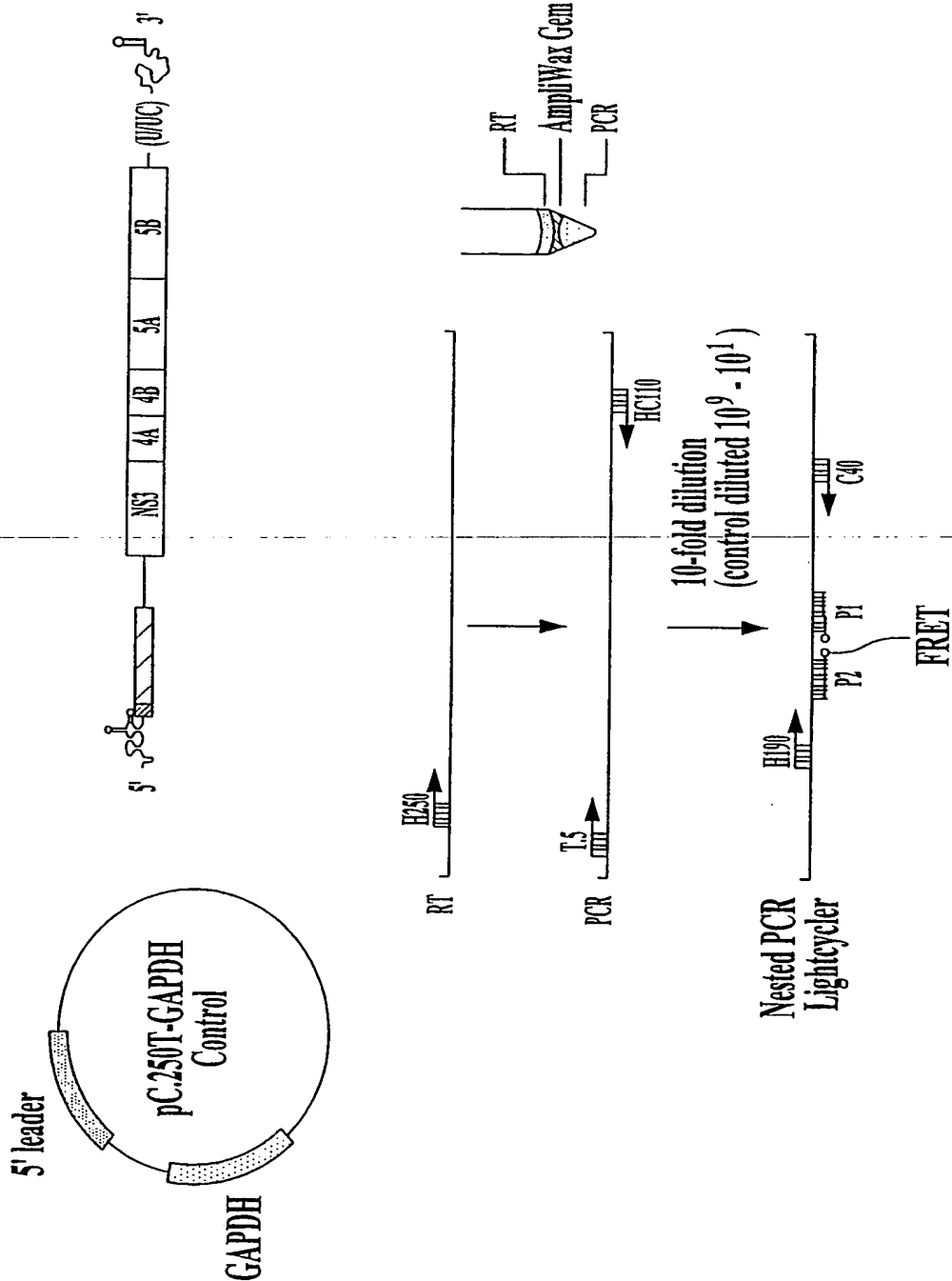


FIG. 30A

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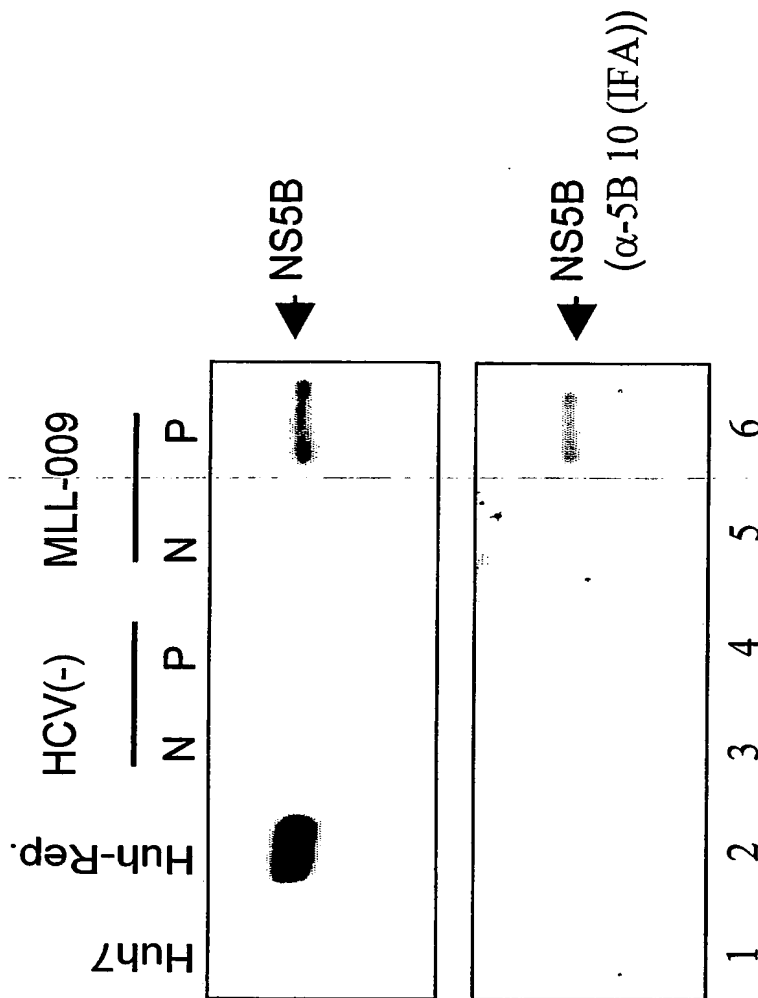
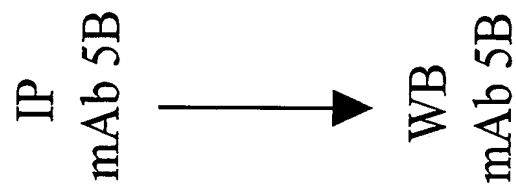


Fig. 30B

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MLL031 +MLL 032
HCV(+)

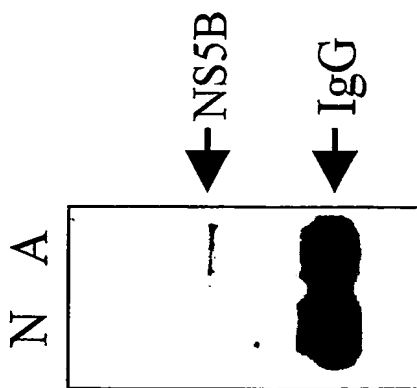


FIG - 31

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Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core

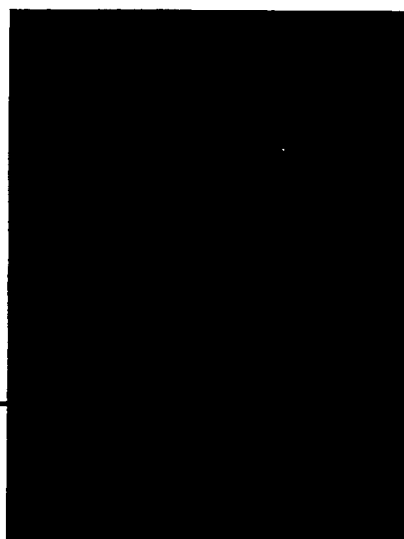
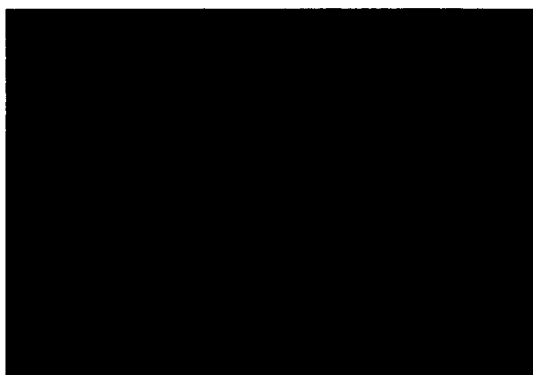


FIG. 32

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Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core



FIG. 33

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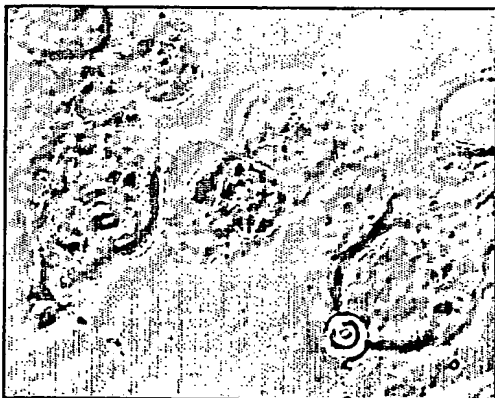
Anti-Core



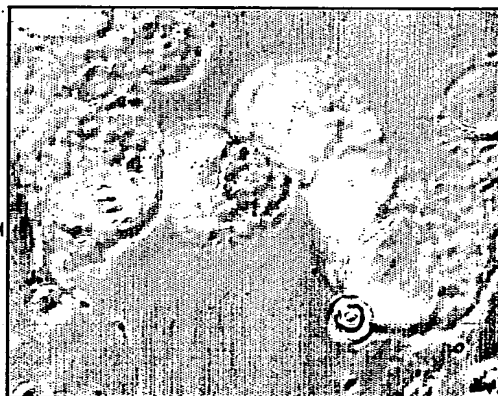
Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core

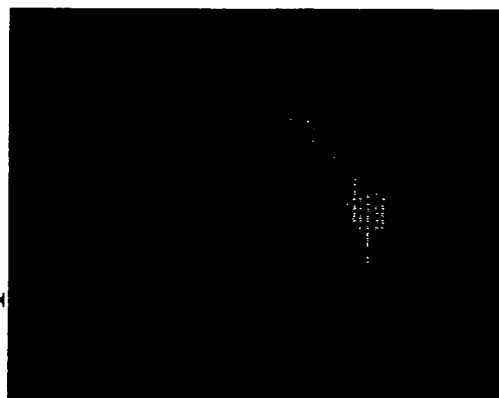


FIG. 34

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Phase



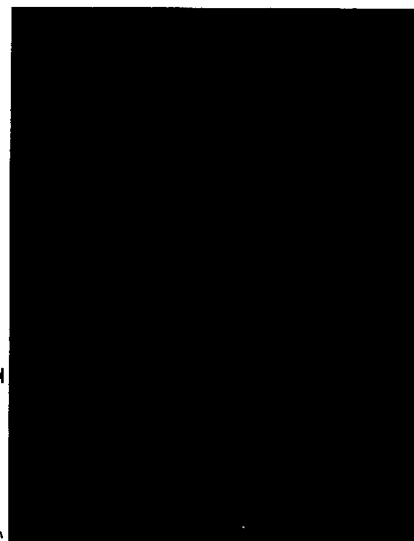
Dapi



Anti-Core



Dapi/Anti-Core



Phase/Dapi/Anti-Core

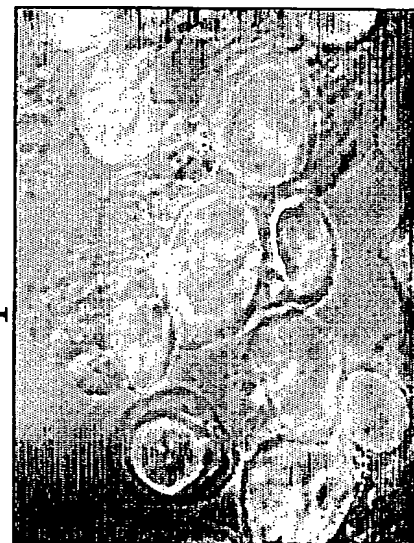


FIG. 35

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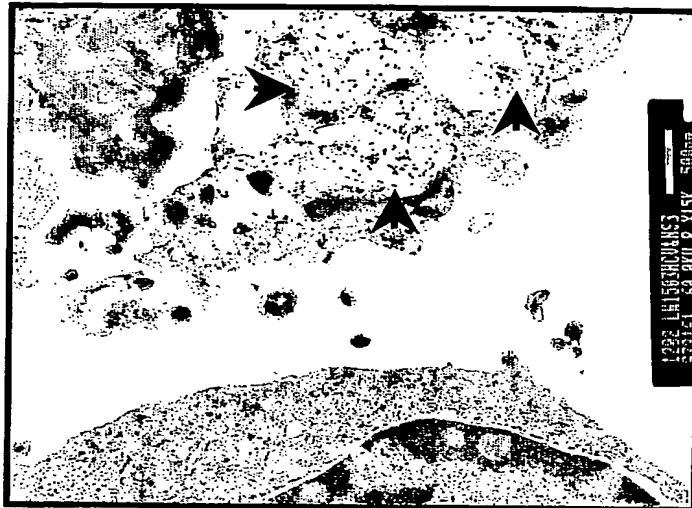


FIG. 36C



FIG. 36B



FIG. 36A

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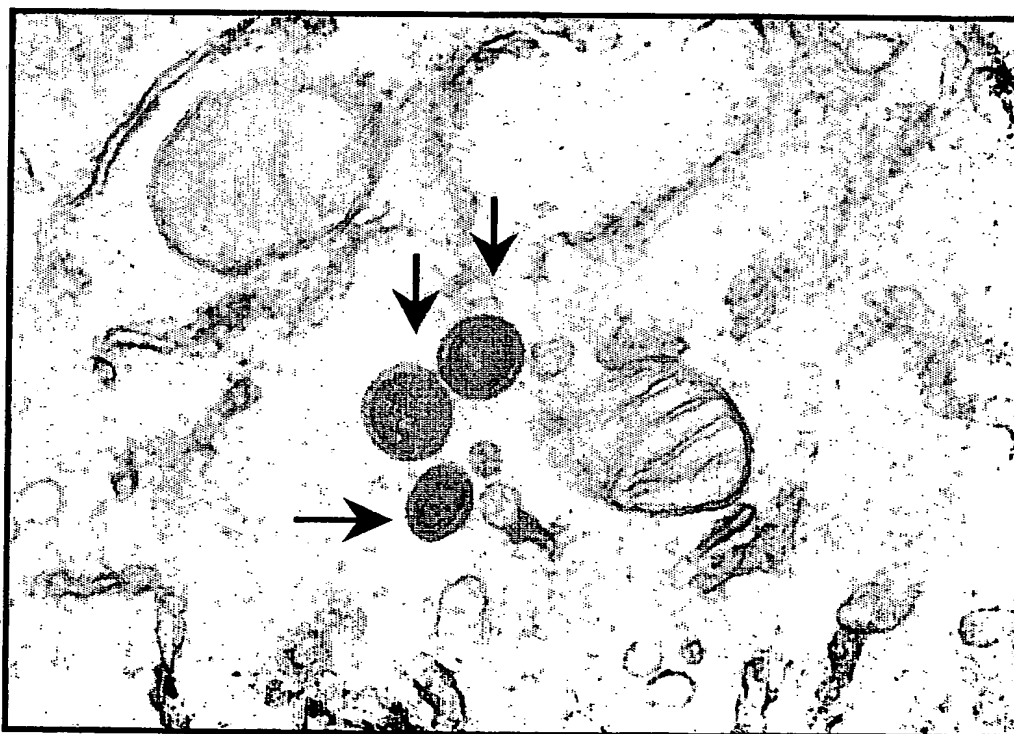
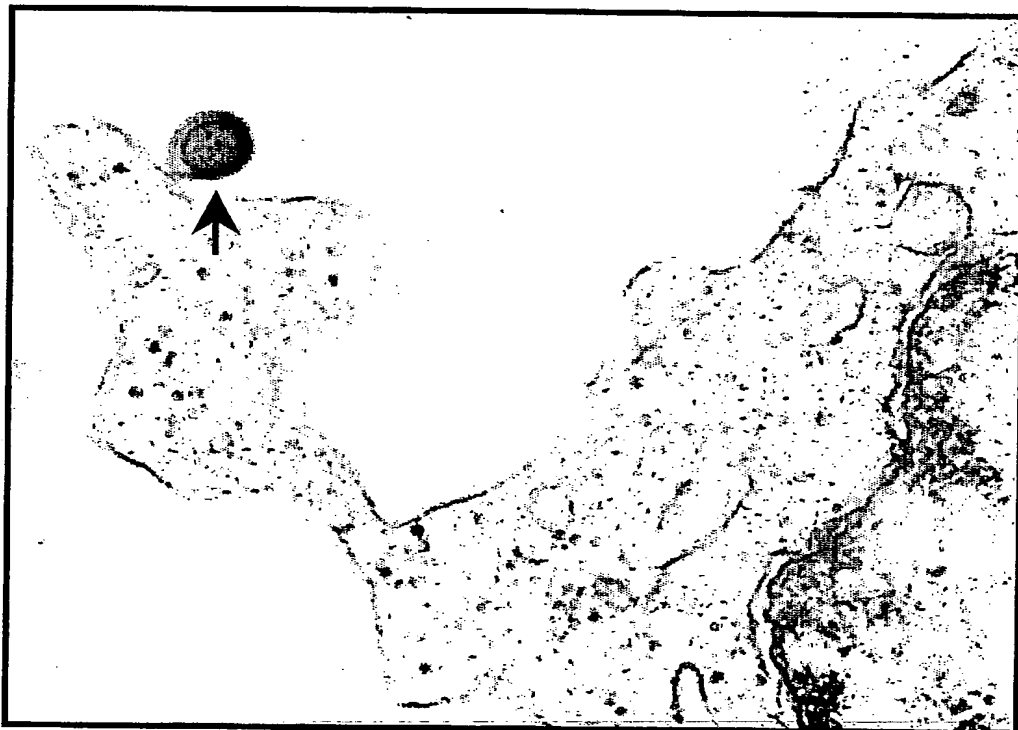


Fig. 37

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Virus partial purification.

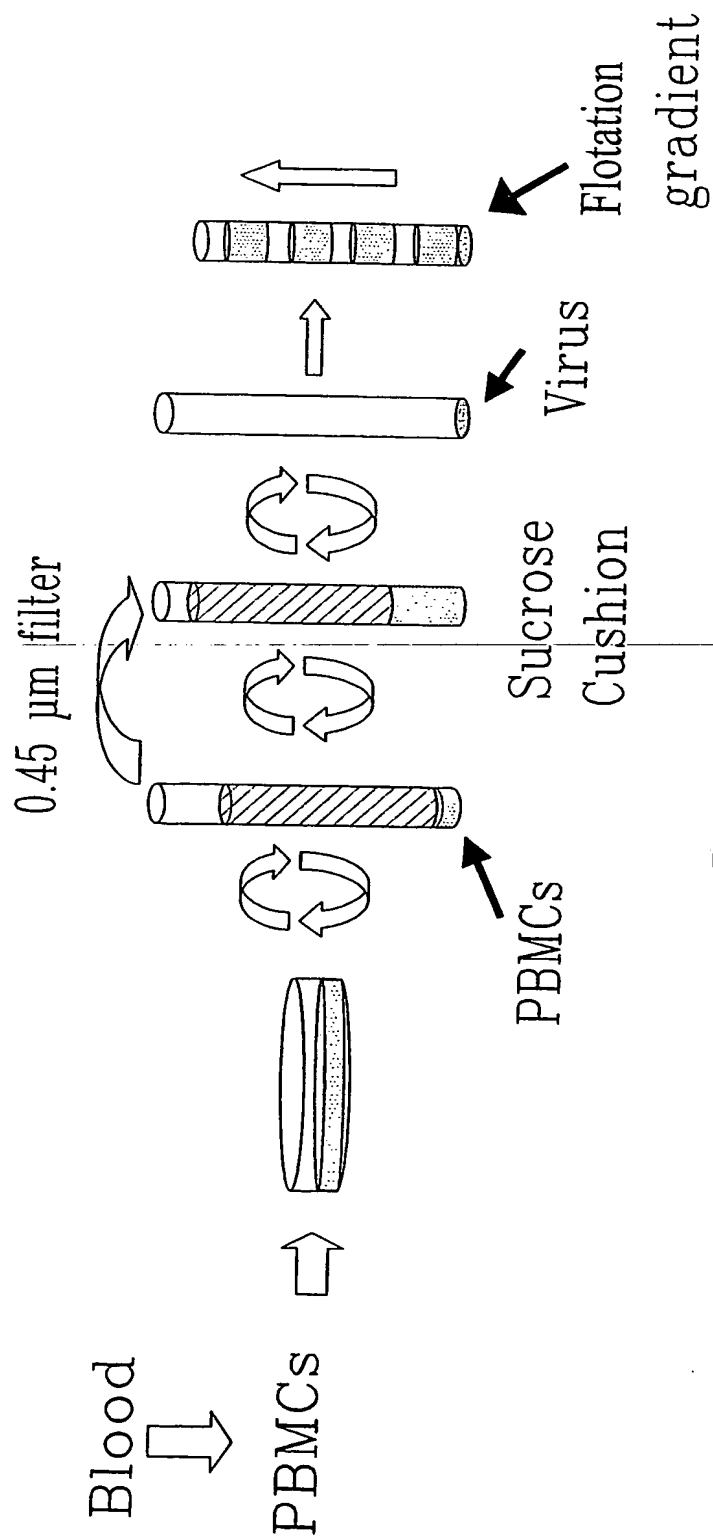


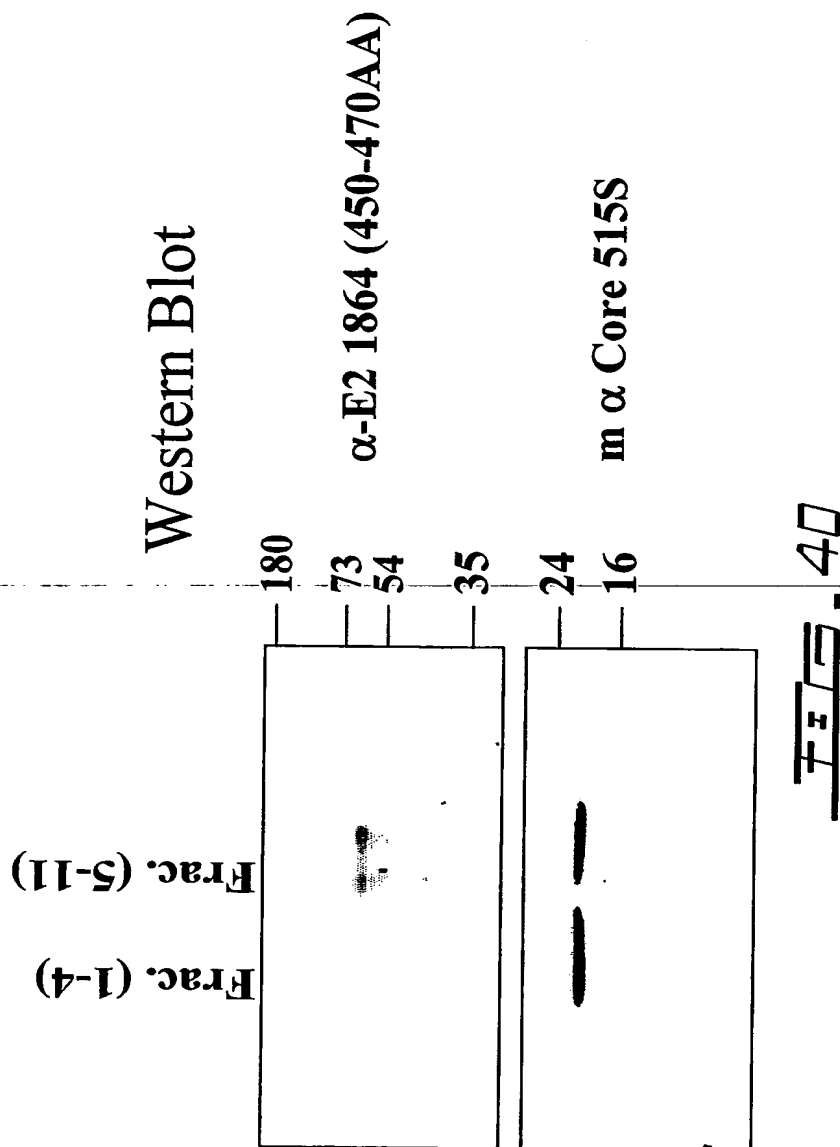
FIG. 3B

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Density Range (g/ml)	Source	Reference
1.15-1.20	HCV-LP in VSV vector	J.Virol (2002) 76, 12325.
1.14-1.18	HCV-LP in insect cells	J. Virol (1998) 72, 3827.
1.12-1.17	Plasma chimps	J. Gen.Virol (1994) 75, 1755
1.09-1.21	Plasma chimps	J.Med.Virol (1991), 34, 206.
1.13-1.17	Plasma chimps	J.Virol (1993) 67, 1953
1.063-1.21	Serum infected donors	J Med Virol (2002) 68, 335
1.11-1.215	HCV(+) PBMCs	-----

7370.39

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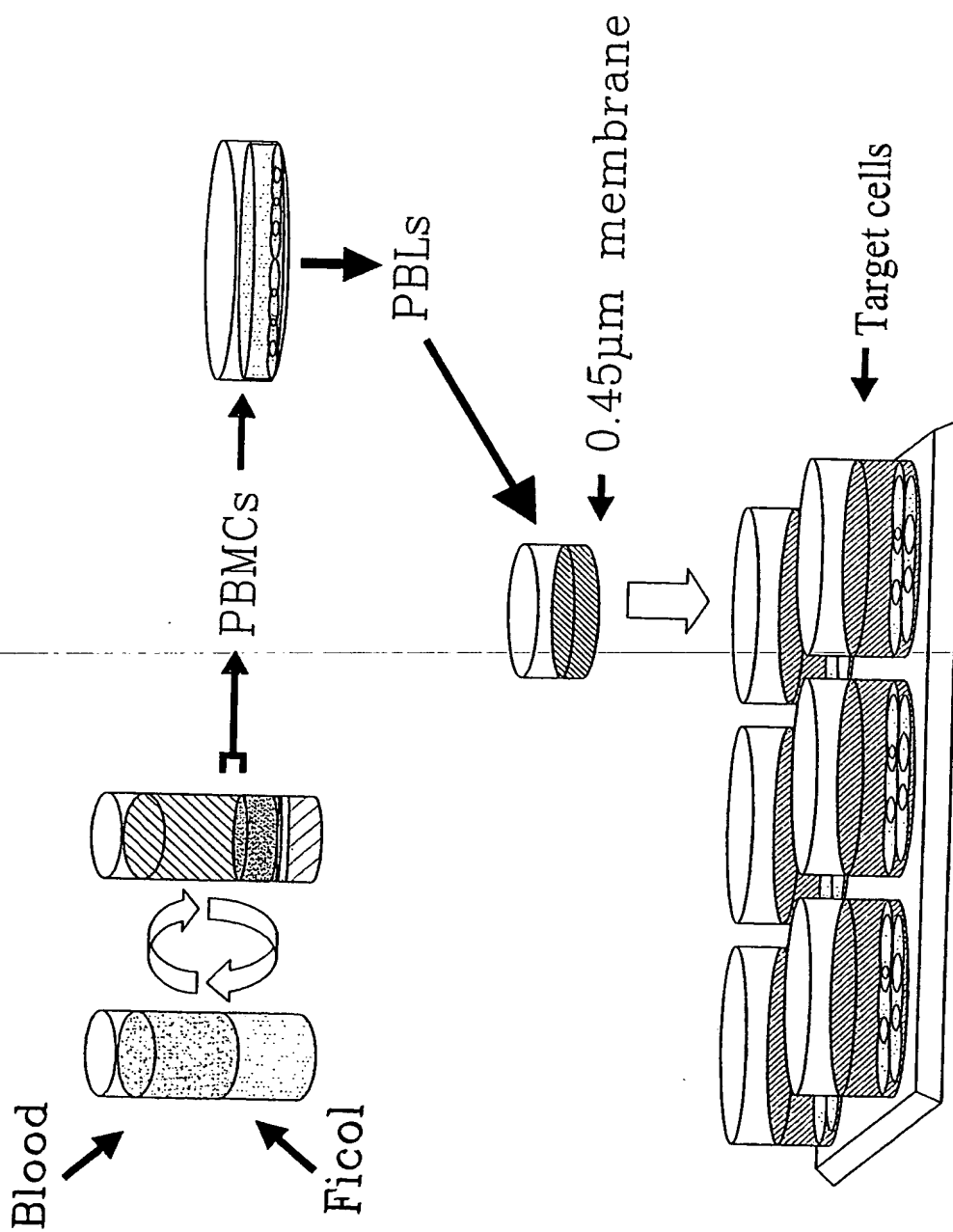
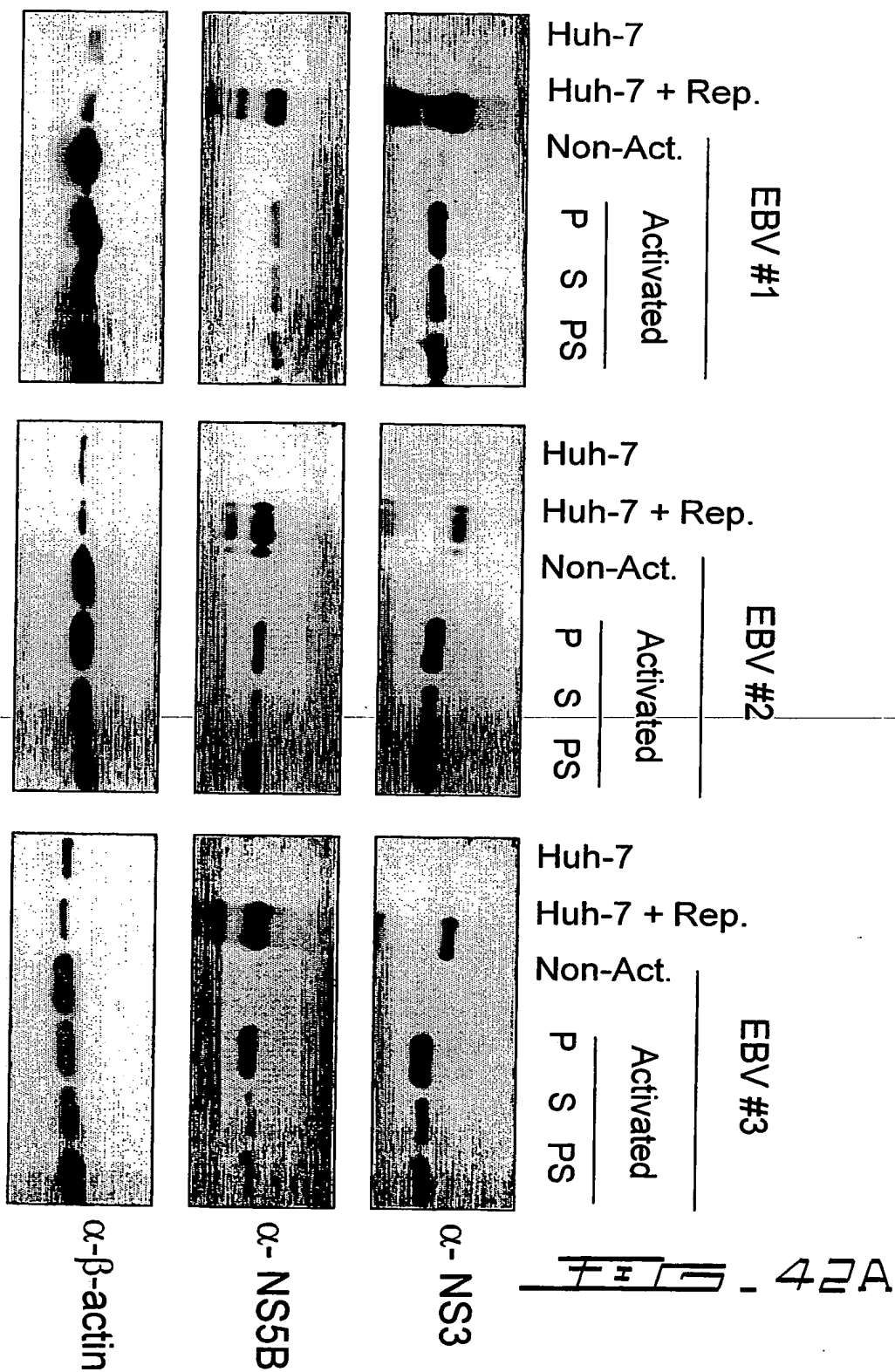
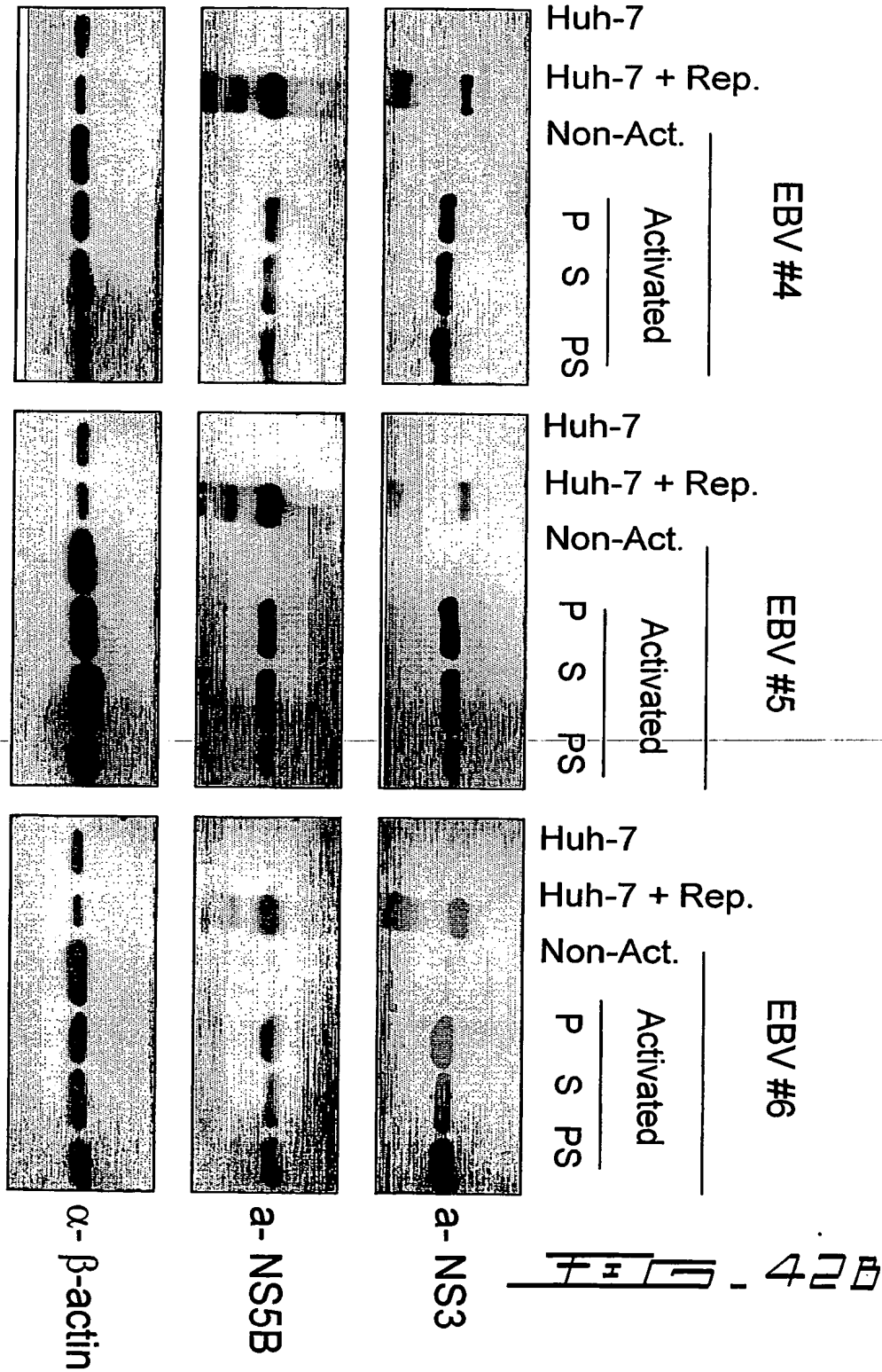


Fig. 41

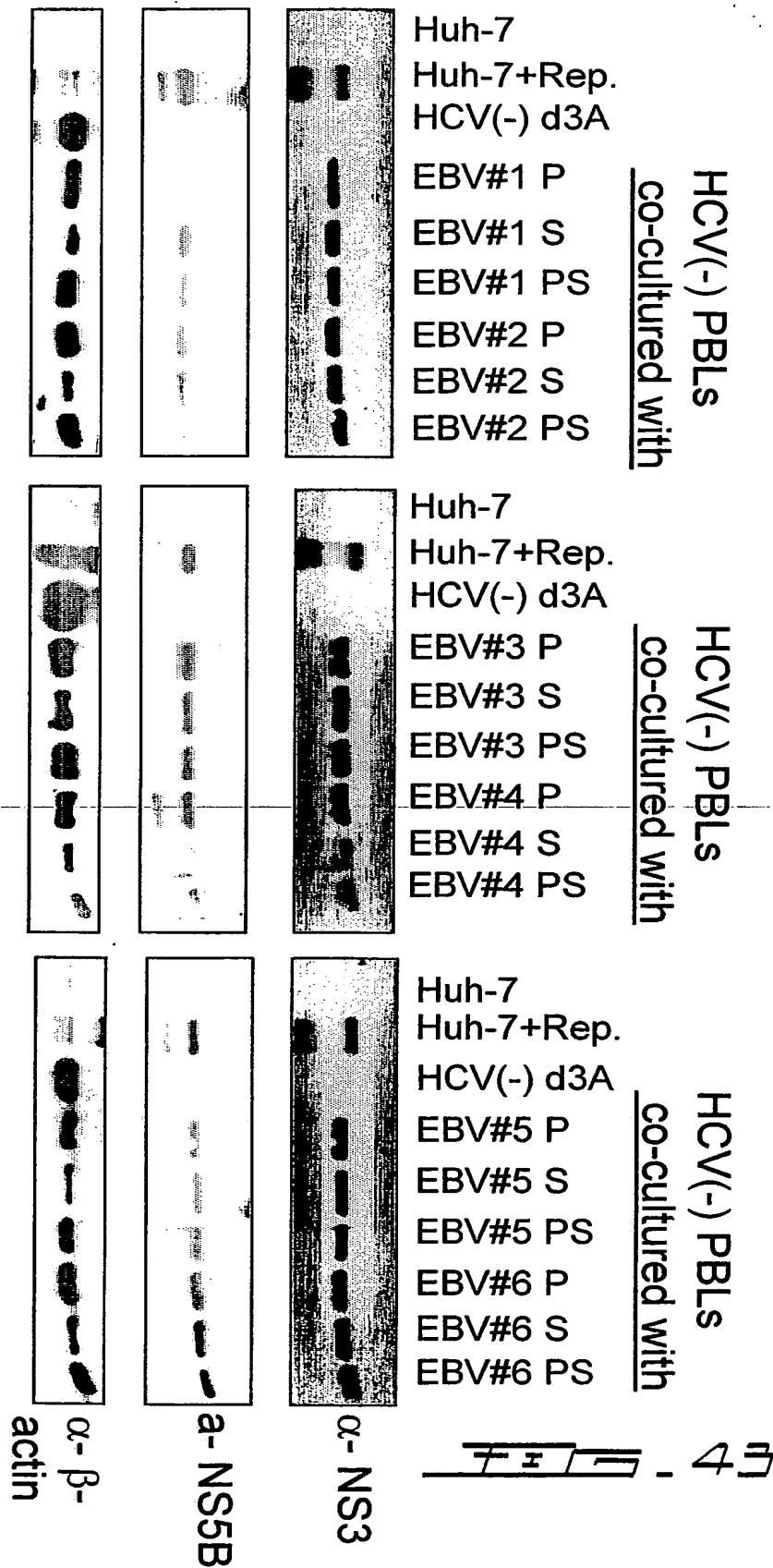
50/72



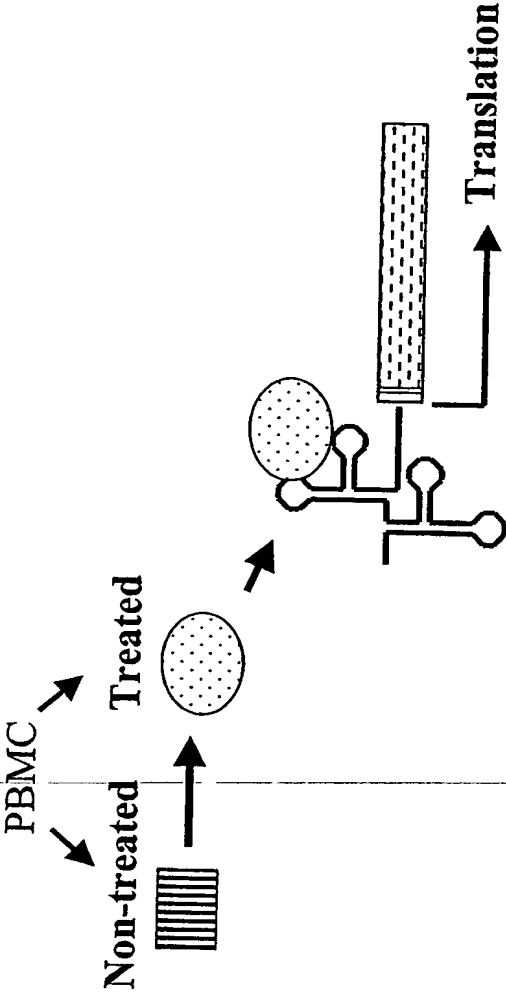
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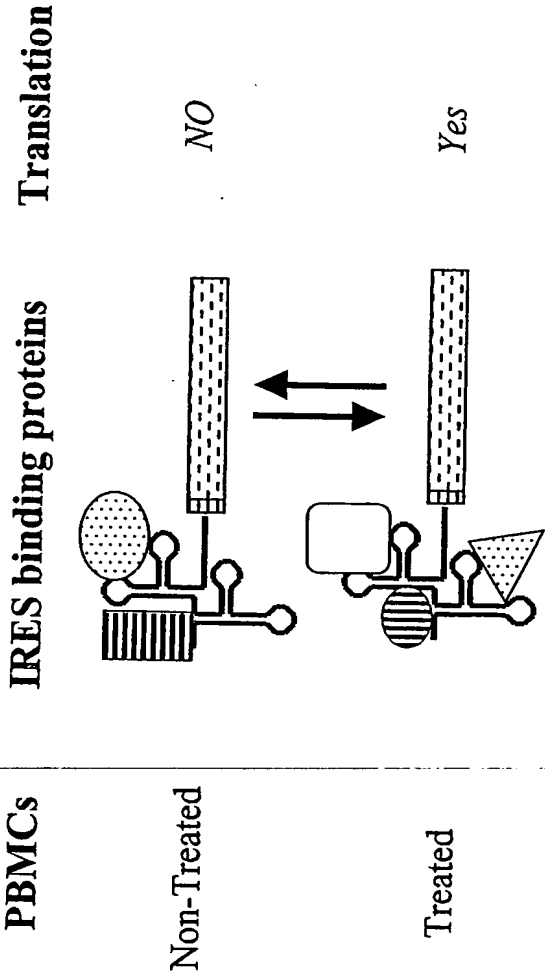
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I- Translation Activator.



II- Translation inhibitor.



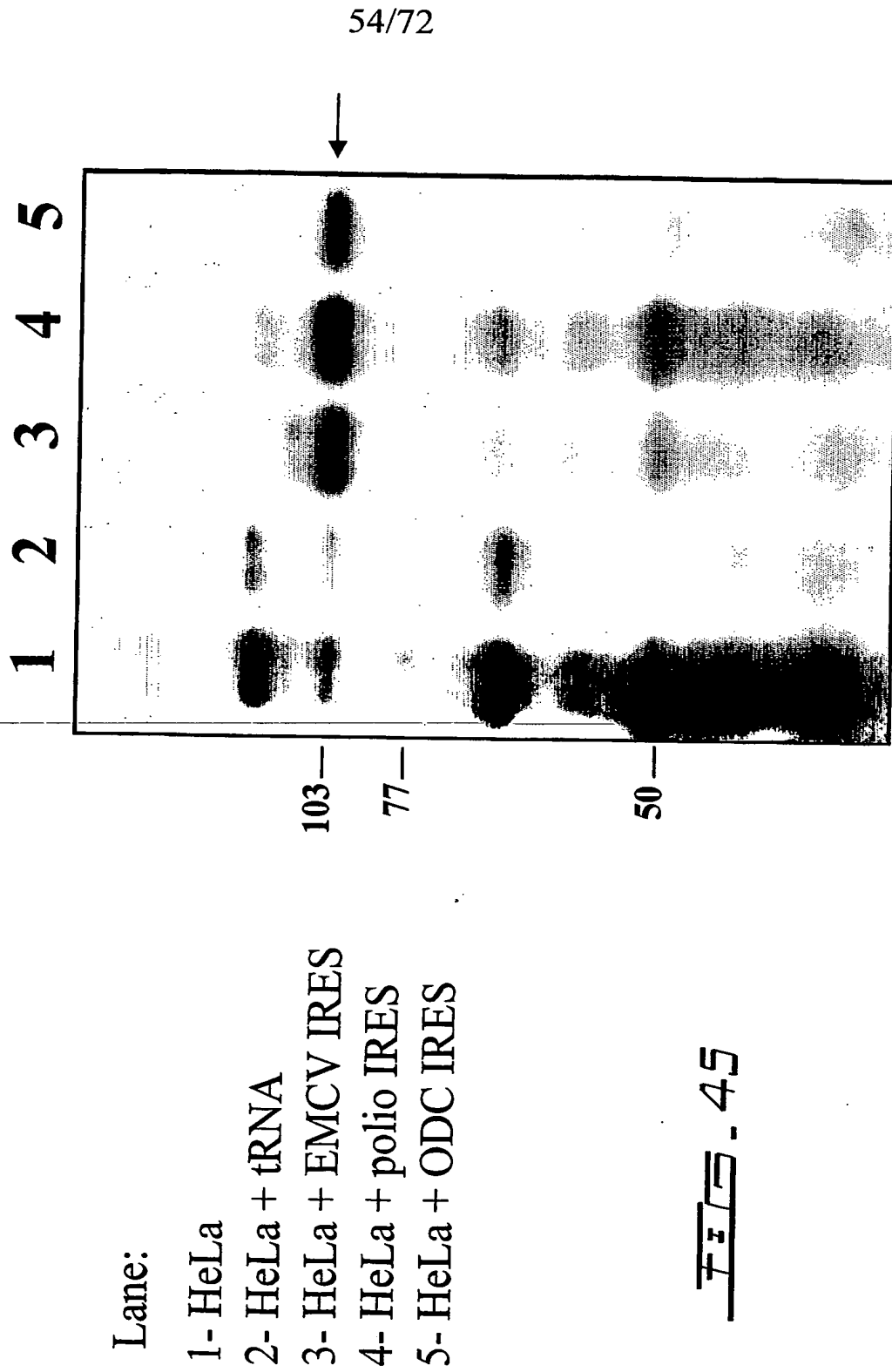
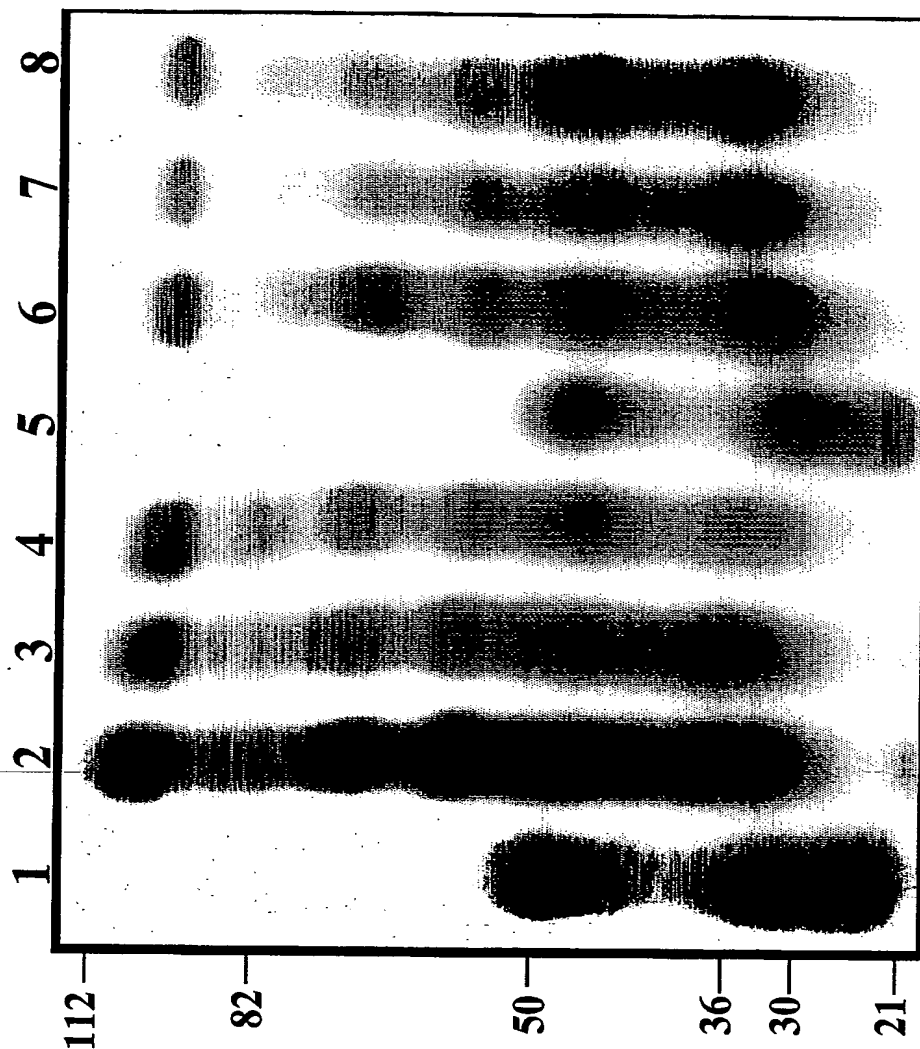


Fig. 45

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Lane:

1- PBMCs NT

2- PBMCs treatment 1

3- PBMCs treatment 2

4- PBMCs treatment

2+DEVA

5- PBMCs NT + HIV

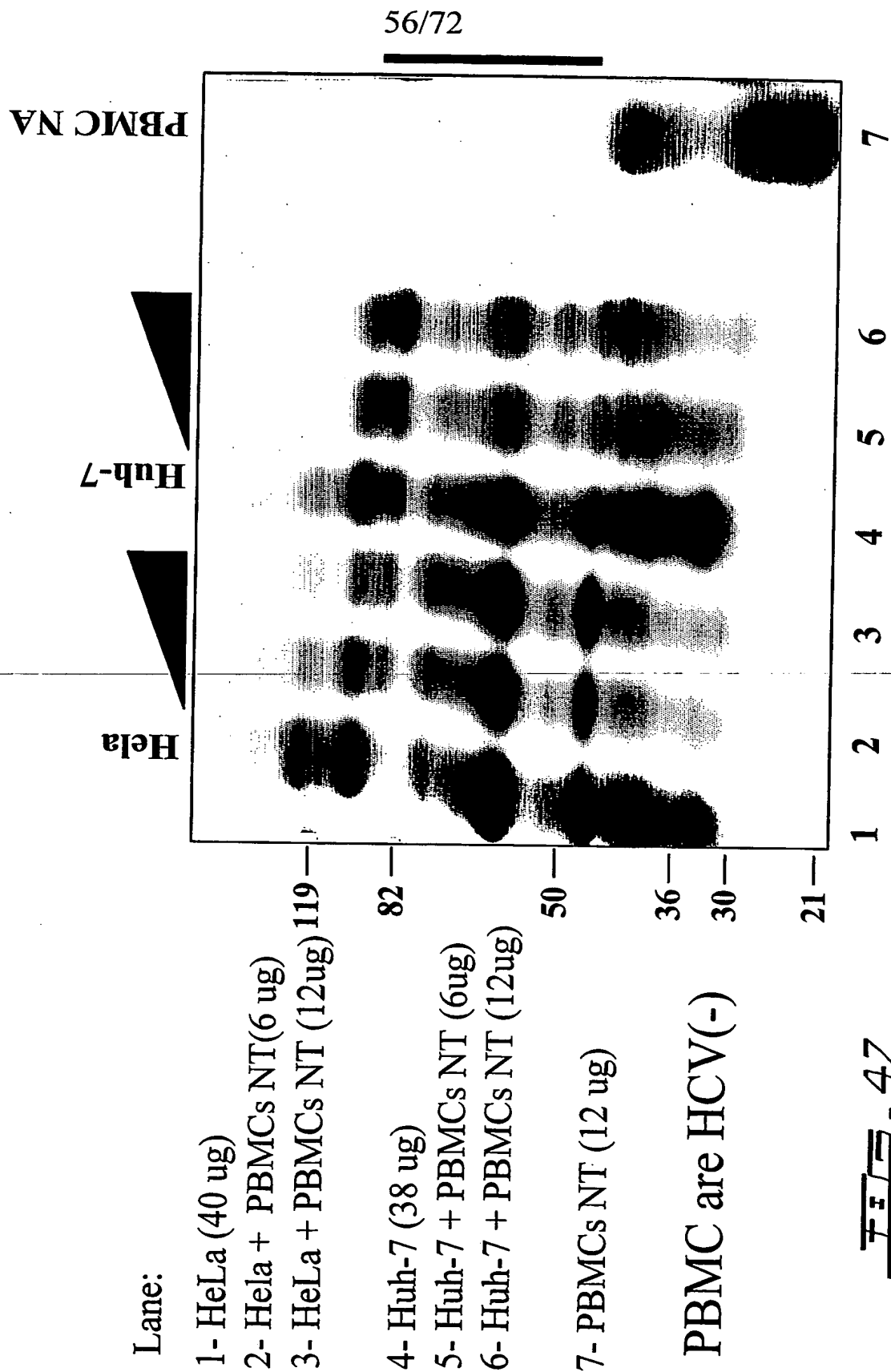
6- PBMCs treatment 1 +HIV

7- PBMCs treatment 2 +HIV

8- PBMCs treatment 2

+DEVA+HIV

Fig. 45



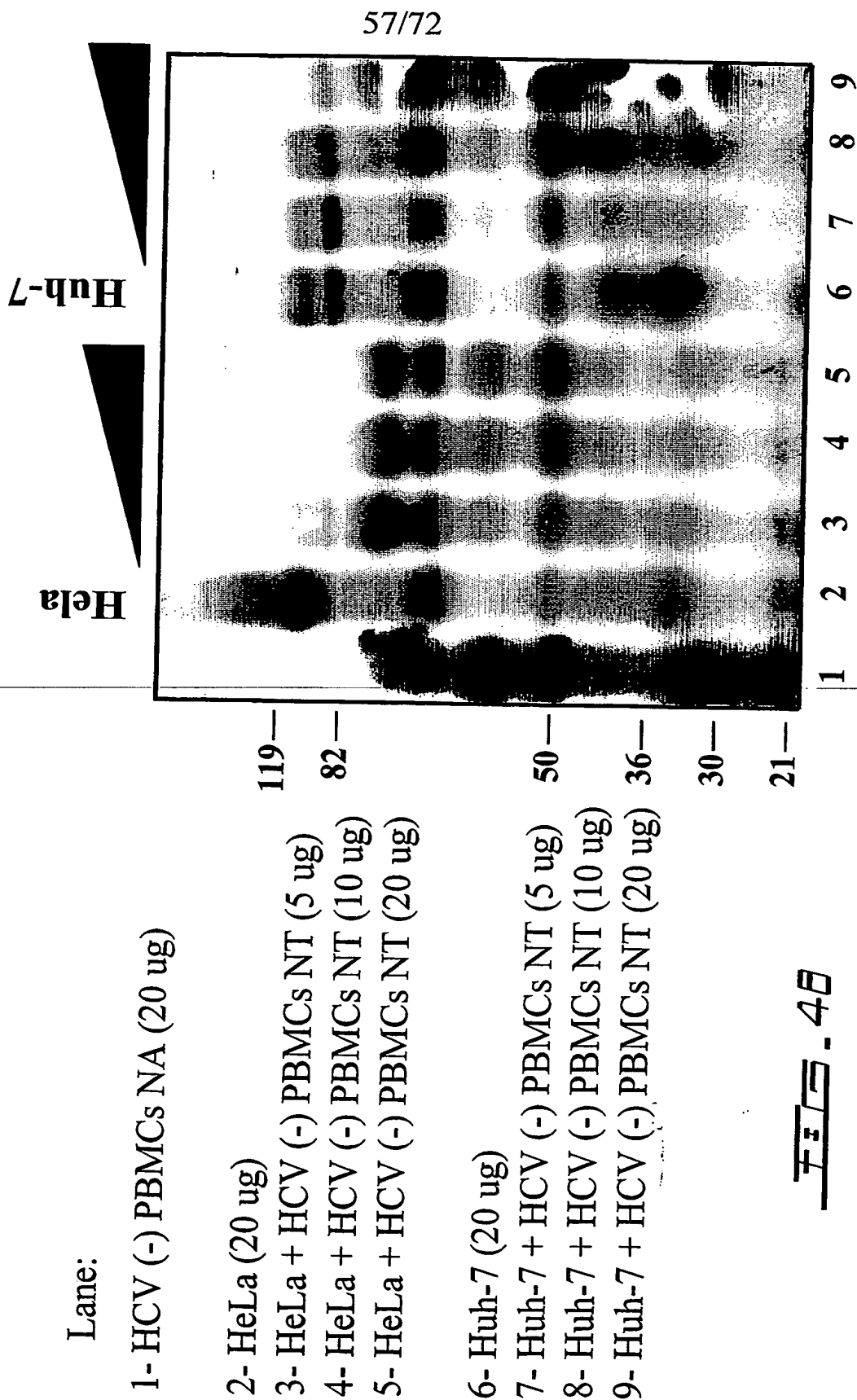
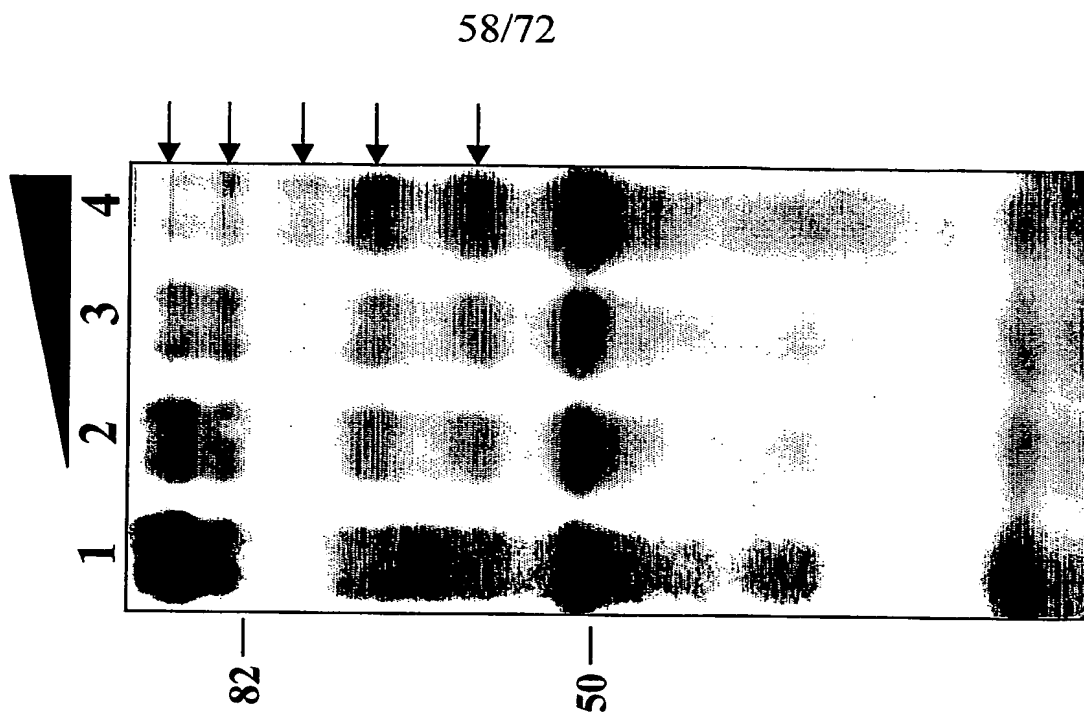


FIG. 4B

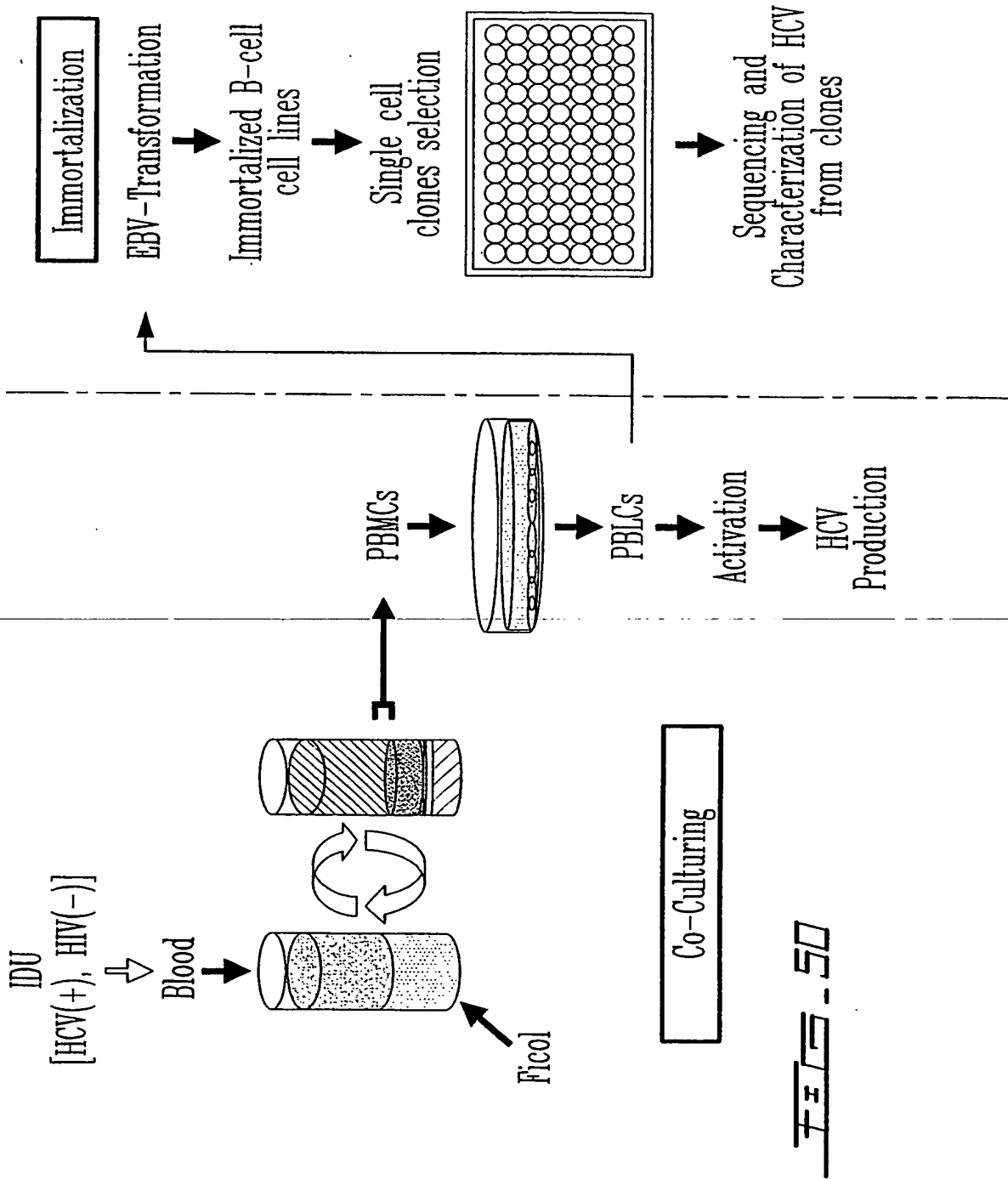


Lane:

- 1- Huh-7 (20ug)
- 2- Huh-7 + HCV (-) PBMCs NT (5ug)
- 3- Huh-7 + HCV (-) PBMCs NT (10ug)
- 4- Huh-7 + HCV (-) PBMCs NT (20ug)

Fig. 49

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HCV(+)-EBV-Transformed B-Cells.

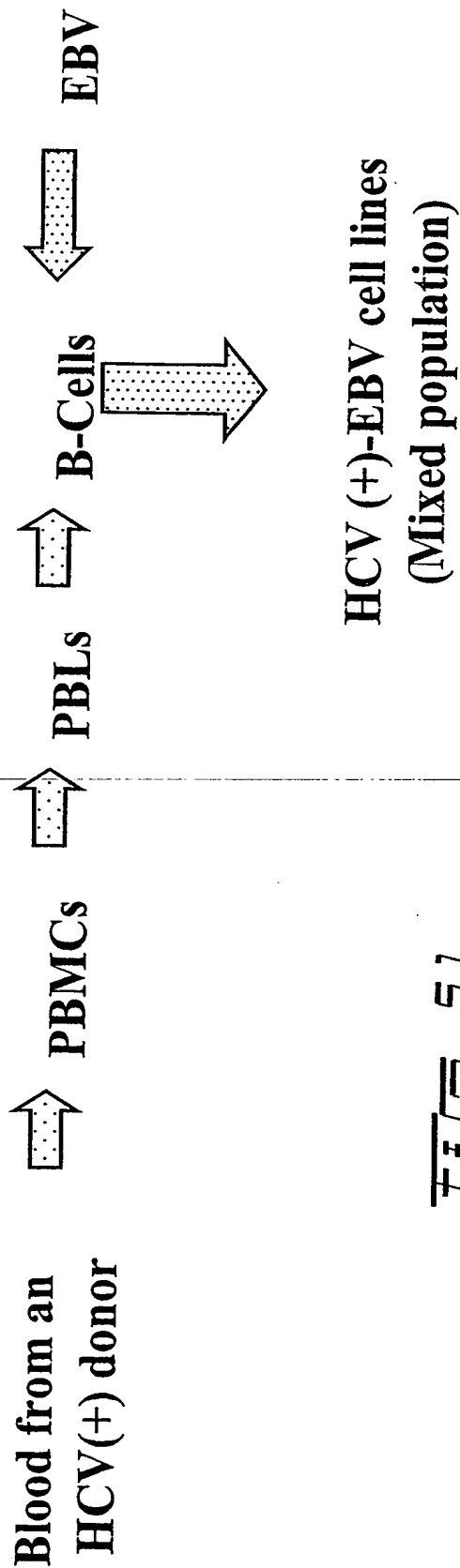


FIG. 51

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HCV RNA is detected in mixed population of EBV-transformed B-cells

HCV (+) Strand RNA

Cell line	Non-Stimulated cells		Stimulated cells	
	RNA Copies /10 ⁶ cells		RNA Copies /10 ⁶ cells	
EBV-1	4.66x10 ⁵		2.33x10 ⁶	
EBV-2	2.77x10 ⁵		7.91x10 ⁴	
EBV-3	3.96x10 ⁶		4.02x10 ⁵	
EBV-4	2.03x10 ⁶		1.57x10 ⁶	
EBV-6	1.41x10 ⁶		4.32x10 ⁵	
EBV-HCV (-)	0		0	

GAPDH mRNA

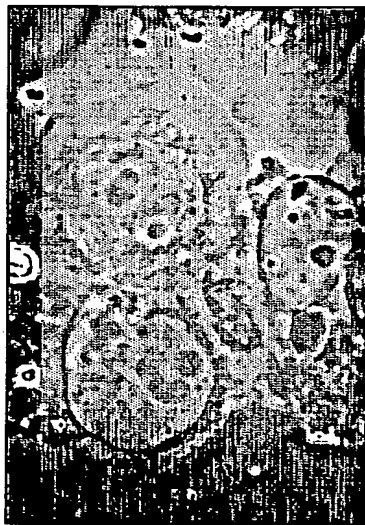
Cell line	Non-Stimulated cells		Stimulated cells	
	RNA Copies /10 ⁶ cells		RNA Copies /10 ⁶ cells	
EBV-1	2.23x10 ⁸		2.19x10 ⁸	
EBV-2	8.73x10 ⁸		2.25x10 ⁸	
EBV-3	1.83x10 ⁹		1.77x10 ⁹	
EBV-4	5.48x10 ⁸		3.79x10 ⁸	
EBV-6	1.26x10 ⁹		9.42x10 ⁸	
EBV-HCV (-)	9.27x10 ⁷		3.62x10 ⁸	

FIG - 52

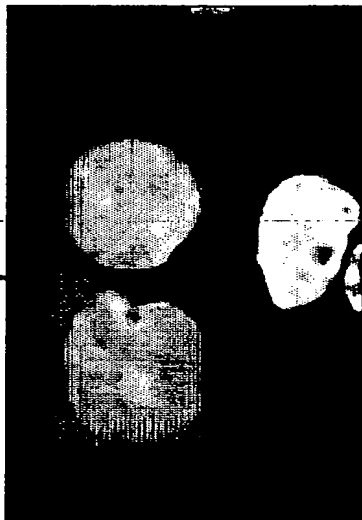
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Control EBV-HCV (-); anti-Core

Phase



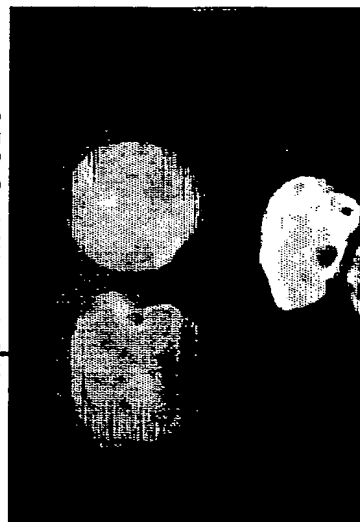
Dapi



Anti-Core



Dapi/Anti-Core



Phase/Dapi/Anti-Core



FIG. 53A

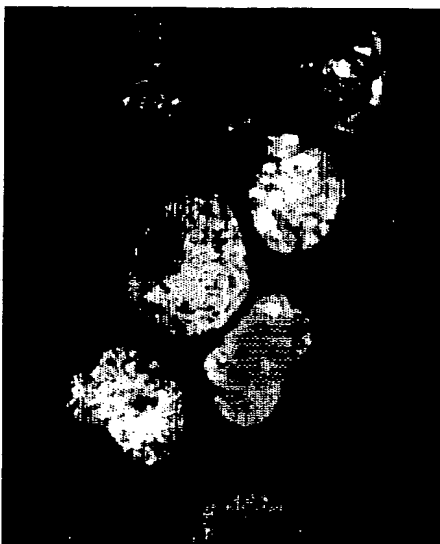
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Detection of Core in EBV-2

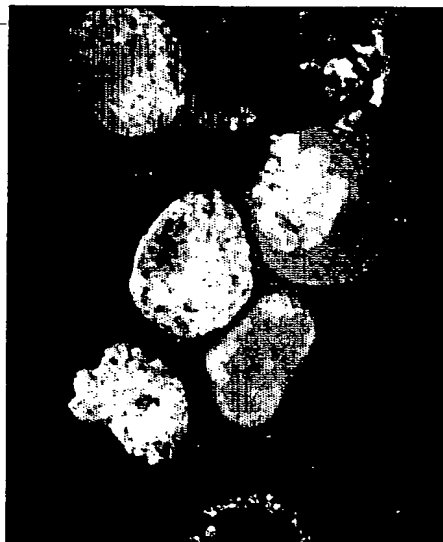
Phase



Anti-Core



Dapi/Anti-Core



Phase/Dapi/Anti-Core



FIG. 53B

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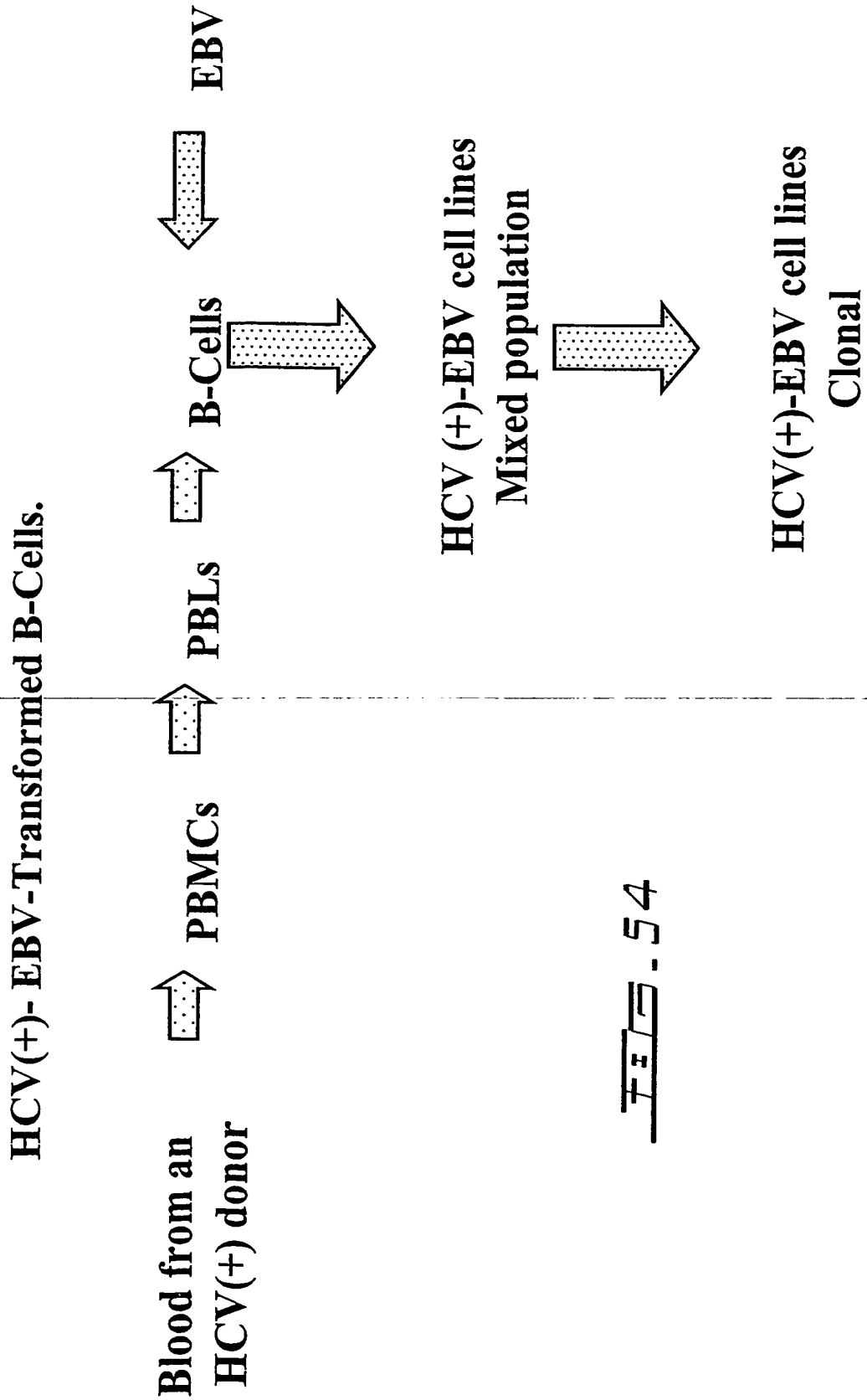
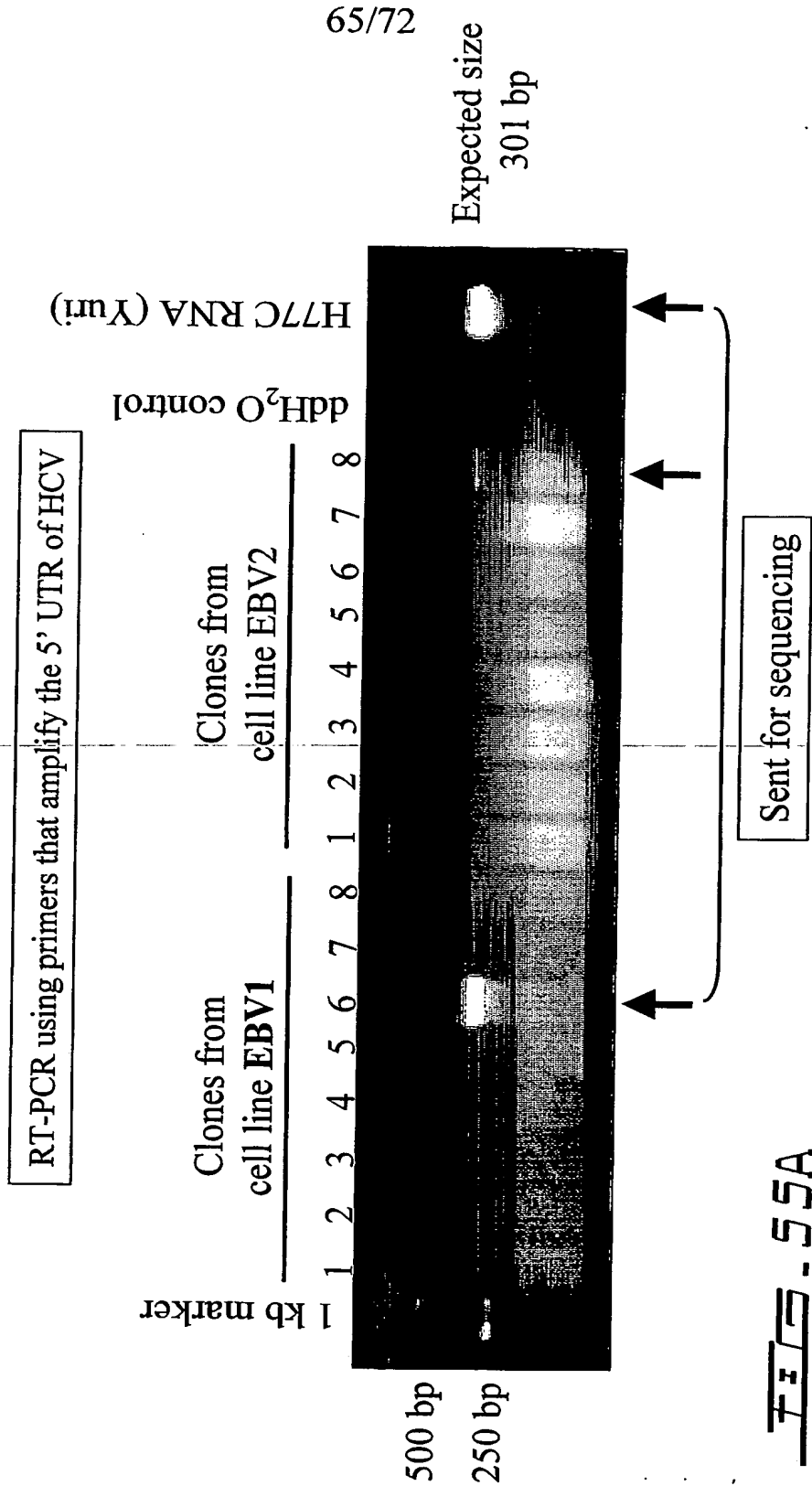


FIG. 54



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**Alignment: H77C (RT-PCR positive control) sequence (top)/
EBV1 clone 6 sequence (bottom)**

CACTCCCCTGTGAGGA	ACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT	
CACTCCCCTGTGAGGA	ACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT	
TAGTATGAGTGTGTCG	TCCAGCCTCCAGGACCCCCCTCCCGGAGAGCCATAGTGTC	
TAGTATGAGTGTGTCG	TCCAGCCTCCAGGACCCCCCTCCCGGAGAGCCATAGTGTC	
TGCGGAACCGGTGAGT	ACACCGGAATTGCCAGGACCGGGTCCTTTCTTGGATAA	<u>G</u>
TGCGGAACCGGTGAGT	ACACCGGAATTGCCAGGACCGGGTCCTTTCTTGGATTAA	
ACCCGCTCA	CA <u>T</u> GCCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAG	
ACCCGCTCA	-ATGCCCTGGAGATTGGGCGTGCCCCCGC <u>G</u> AGACTGCTAGCCGAGTAG	
TGTTGGGTCGCGAAAG	GCCTTGTTGGTACTGCCTGATAGGGT	
TGTTGGGTCGCGAAAG	GCCTTGTTGGTACTGCCTGATAGGGT	

Blue: sequence from virus in the serum (MLL-005).

77C-55B

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Alignment: H77C (RT-PCR positive control) sequence (top)/
EBV2 clone 8 sequence (bottom).

CCAGGACCCCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC
CCAGGACCCCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC

GGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGG
GGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGG

ATAAACCCGCTCAATGCCCTGGAGATTGGGCGTGCCCCCGCAAG
ATAAATCCGCTCAATGCCCTGGAGATTGGGCGTGCCCCCGCAAG

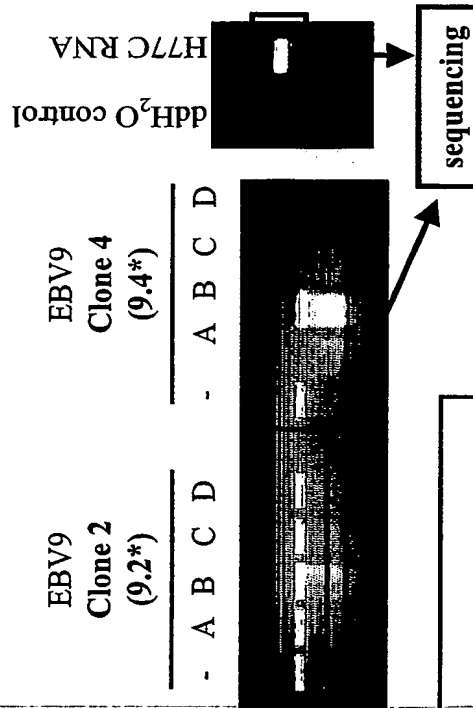
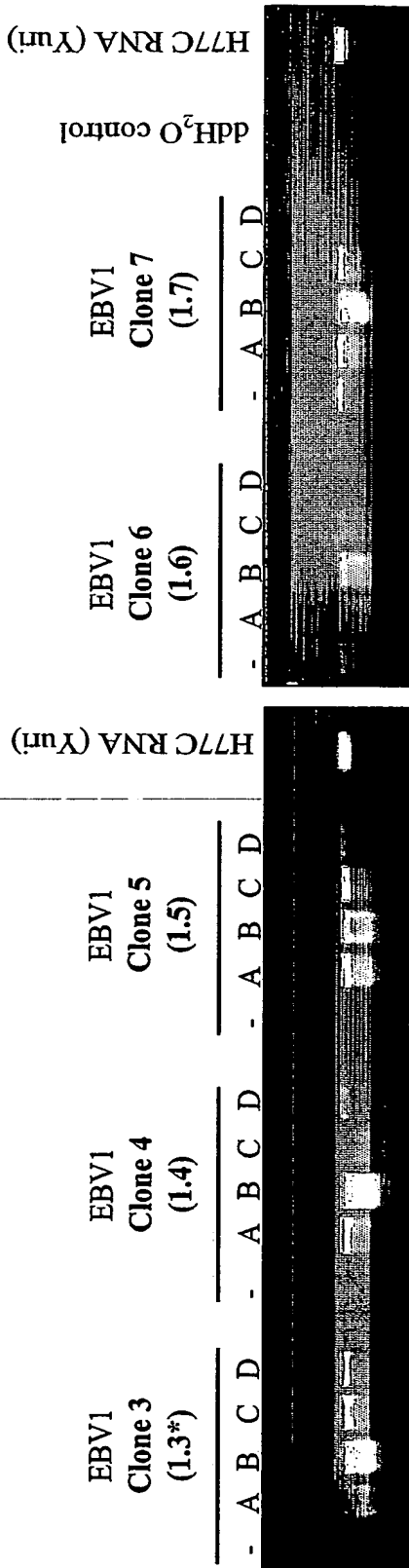
ACTGCTAGCCGAGTAGTGTGGTCCGAAAGGCCTTGTTGGTAC
ACTGCTAGCCGAGTAGTGTGGTCCGAAAGGCCTTGTTGGTAC

TGCCTGATAGGTGCTTGCGAGTGCCCCGGAGGTCTCGTAGAC
TGCCTGATAGGTGCTTGCGAGTGCTCCGGGAGGTCTCGTAGAC

CGTGCA
CGTGCA

File - 55C

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- = clone alone (not diluted with other cells)
 A= diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)
 B= diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)
 C= diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)
 D= diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

7-17-56

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Alignment of all 9.2 sequences

H77C CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
 9.2 final seq CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
 9.2a final seq CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
 9.2b final seq CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
 9.2c final seq CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT
 9.2d final seq CACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCT

H77C AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2 final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2a final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2b final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2c final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2d final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC

H77C CCTCCCGGGAGAGCCATAGTGTCTGCGGAACCGGTGAGTACAC
 9.2 final seq CCTCCCGGGAGAGCCATAGTGTCTGCGGAACCGGTGAGTACAC
 9.2a final seq CCTCCCGGGAGAGCCATAGTGTCTGCGGAACCGGTGAGTACAC
 9.2b final seq CCTCCCGGGAGAGCCATAGTGTCTGCGGAACCGGTGAGTACAC
 9.2c final seq CCTCCCGGGAGAGCCATAGTGTCTGCGGAACCGGTGAGTACAC
 9.2d final seq CCTCCCGGGAGAGCCATAGTGTCTGCGGAACCGGTGAGTACAC

- = clone alone (not diluted with other cells)

a= diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)

b= diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)

c= diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)

d= diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

Red= Variation with respect to clone 9.2

~~718~~ - 57A

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Alignment of all 9.2 sequences

H77C	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCT
9.2 final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCT
9.2a final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGAT <u>T</u> AACCCGCT
9.2b final seq	CGGAATTGCC <u>CGG</u> A <u>G</u> AC <u>T</u> GGGTCCTTTCTTGGATAAACCC <u>A</u> CT
9.2c final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCT
9.2d final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGAT <u>T</u> AATCCGCT
H77C	CAATGCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2 final seq	CAATGCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2a final seq	CAATGCCTGGAGATTGGGCGTGCCCCCGC <u>G</u> AGACTGCTAGCCG
9.2b final seq	C <u>T</u> A <u>T</u> GCCCGG <u>C</u> CATTTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2c final seq	CAATGCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2d final seq	CAATGCCTGGAGATTGGGCGTGCCCCCGC <u>G</u> AGACTGCTAGCCG

FIG. 57B

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Alignment of all 9.2 sequences

H77C	AGTAGTGTGGTCCGAAAGGCCCTTGTTGGTACTGCCTGATAGG
9.2 final seq	AGTAGTGTGGTCCGAAAGGCCCTTGTTGGTACTGCCTGATAGG
9.2a final seq	AGTAGTGTGGTCCGAAAGGCCCTTGTTGGTACTGCCTGATAGG
9.2b final seq	AGTAGC <u>CG</u> TGGGT <u>T</u> GCGAAAGGCCCTTGTTGGTACTGCCTGATAGG
9.2c final seq	AGTAGTGTGGTCCGAAAGGCCCTTGTTGGTACTGCCTGATAGG
9.2d final seq	AGTAGTGTGGTCCGAAAGGCCCTTGTTGGTACTGCCTGATAGG
H77C	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
9.2 final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
9.2a final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
9.2b final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
9.2c final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA
9.2d final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCTGTAGACCGTGCA

FIG. 57B (Cont.)

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Variation found when clone 9.2
is diluted in BJAB cells (---)
or in EBV-Naïve cells (-----)

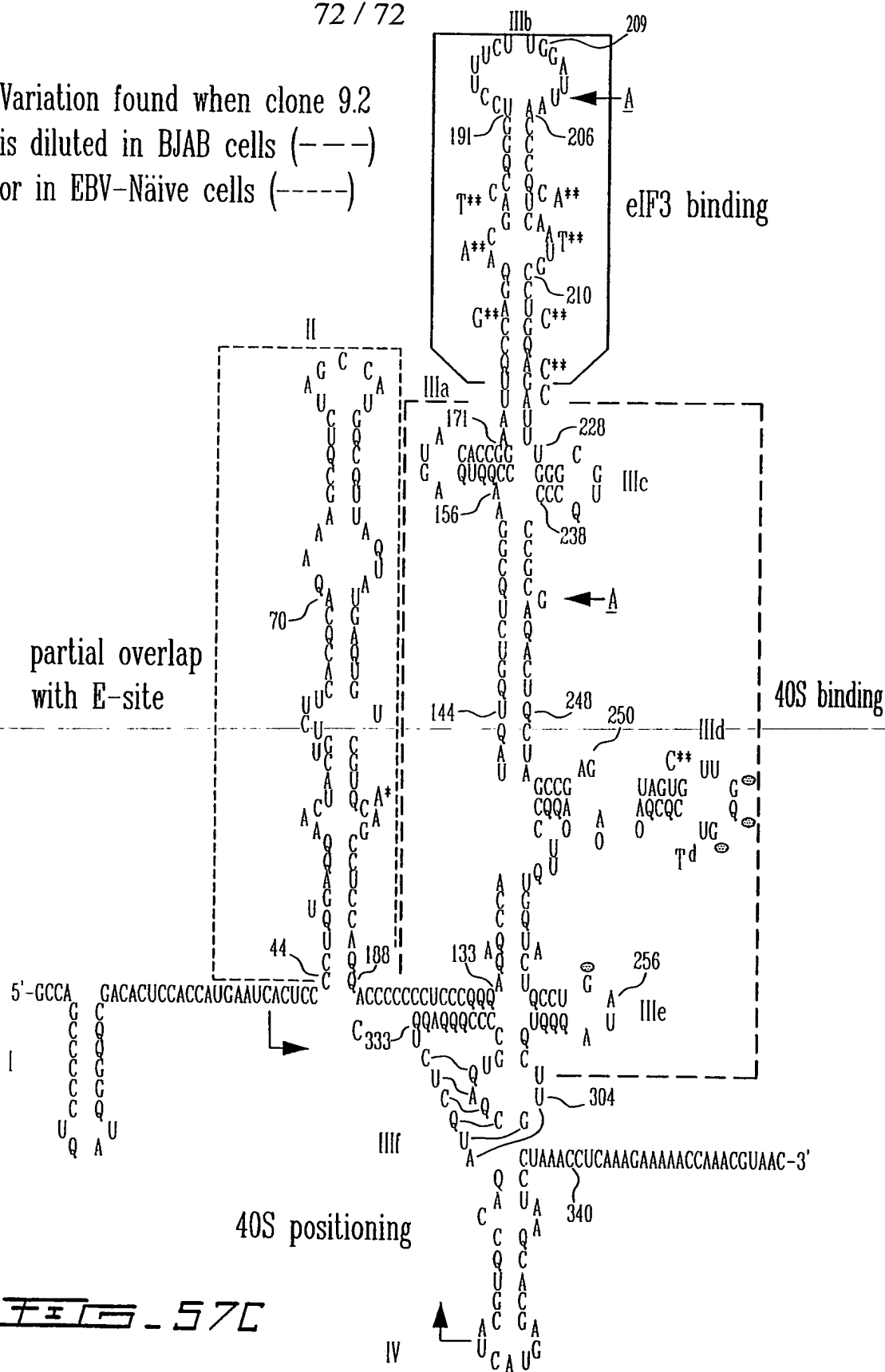


FIG. 57C

➔ = Primers

AUG start codon

IRES structure from Sarnow P (2003), J Virol. 77, 2801-6

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